A STUDY OF EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR(EGFR) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EPITHELIAL OVARIAN NEOPLASMS.

Dissertation submitted in

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CERTIFICATE

This is to certify that this Dissertation entitled **"A STUDY OF EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EPITHELIAL OVARIAN NEOPLASMS"** is the bonafide original work of **Dr. D.KANMANI**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2016.

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DECLARATION

I, **Dr.D.KANMANI,** solemnly declare that the dissertation titled "**A STUDY OF EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR(EGFR) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EPITHELIAL OVARIAN NEOPLASMS"** is the bonafide work done by me at the Institute of pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr.K.RAMA,** M.D., Professor of Pathology,Institute of social obstetrics and Govt. Kasturba Gandhi hospital , Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. D.KANMANI**

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INSTITUTE ETHICS COMMITTEE
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CERTIFICATE OF APPROVAL

To

Dr.D.Kanmani
Post Graduate M.D.(Pathology)
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Dear Dr. D.Kanmani

The Institutional Ethics Committee has considered your request and approved your study titled The Study of the Expression of EGFR (Epidermal growth factor receptor) and VEGF (Vascular Endothelial growth factor) in Epithelial Ovarian Neoplasms No.03102014.
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We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

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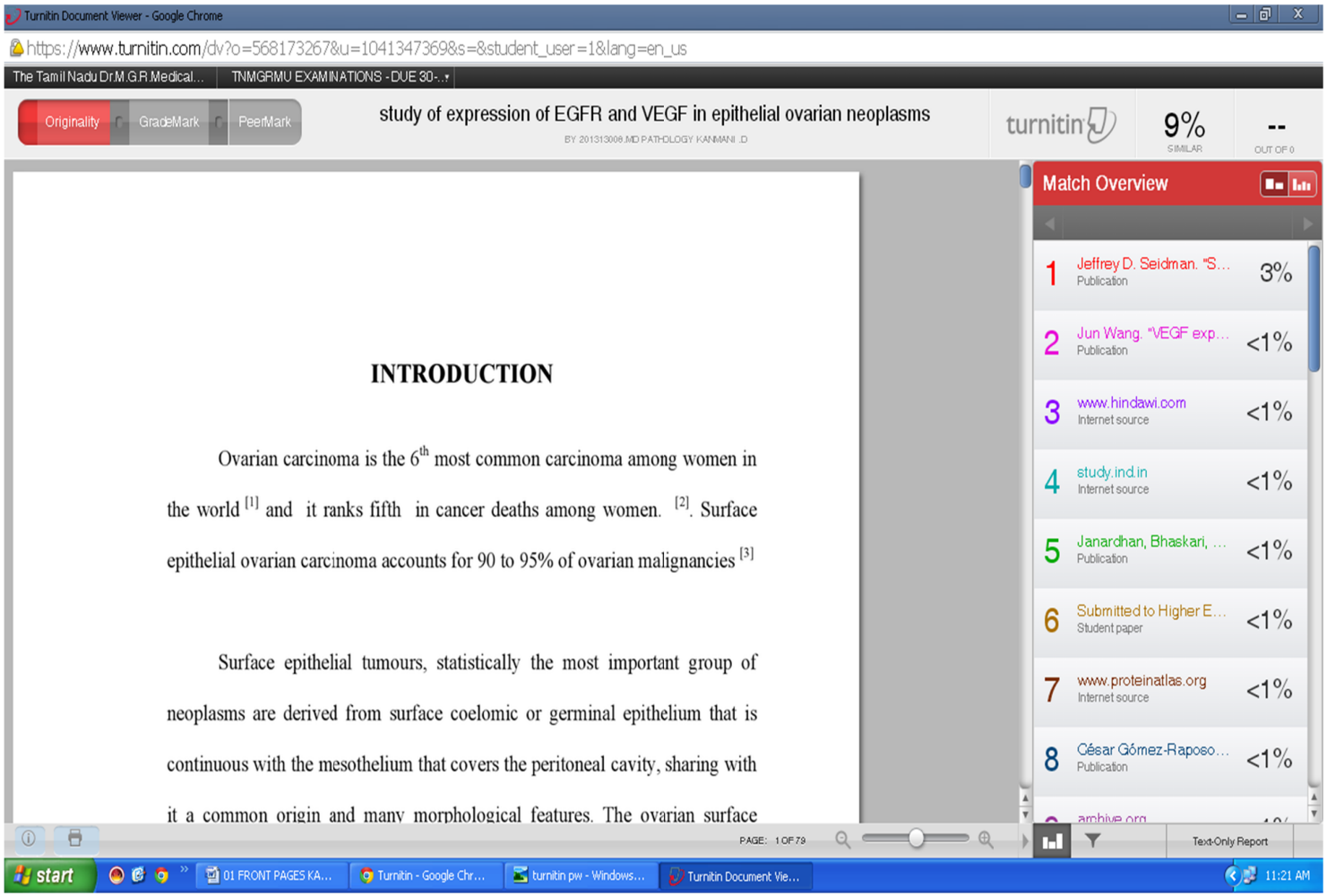
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INTRODUCTION
Ovarian carcinoma is the 6th most common carcinoma among women in the world [1] and it ranks fifth in cancer deaths among women. [2]. Surface epithelial ovarian carcinoma accounts for 90 to 95% of ovarian malignancies [3]
Surface epithelial tumours, statistically the most important group of neoplasms are derived from surface coelomic or germinal epithelium that is continuous with the mesothelium that covers the peritoneal cavity, sharing with it a common origin and many morphological features. The ovarian surface
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INTRODUCTION

Ovarian carcinoma is the 5th most common carcinoma among women in the world [1] and it ranks fifth in cancer deaths among women. [2] Surface epithelial ovarian carcinoma accounts for 80 to 95% of ovarian malignancies [3].

Surface epithelial tumours, statistically the most important group of neoplasms are derived from surface coelomic or germinal epithelium that is continuous with the mesothelium that covers the peritoneal cavity, sharing with it a common origin and many morphological features. The ovarian surface epithelium involved in metaplastic or neoplastic conditions often undergo 'müllerian differentiation' and may produce any of the adult structures formed by the müllerian ducts including tubal, endometrial and endocervical mucosa, singly or in combination [4]. It has also been noted that many of the surface epithelial tumors arise from the invaginated portion of the epithelium that forms surface epithelial glands and cysts [5]. Another proposed origin of some ovarian epithelial tumours especially serous type) is the epithelium of the tubal fimbriae and fimbriae are the most common sites of early serous carcinoma in women with BRAC mutations [6].

1

ABBREVIATIONS

EGFR	:	Epidermal Growth Factor Receptor
VEGF	:	Vascular Endothelial Growth Factor
NCRP	:	National Cancer Registry Programme
WHO	:	World Health Organisation
ICMR	:	Indian Council Of Medical Research
HNPCC	:	Hereditary Non Polyposis Colorectal Cancer
STIC	:	Serous Tubal Intraepithelial Carcinoma
CK	:	CytoKeratin
IHC	:	ImmunoHistoChemistry
CEA	:	CarcinoEmbryonic Antigen
EDTA	:	Ethylene Diamine Tetra Acetic acid
MAPK	:	Mitogen Activated Protein Kinase
H & E	:	Hematoxylin & Eosin
FIGO	:	International Federation of Gynecology and Obstetrics

CONTENTS

A STUDY OF EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR(EGFR) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN EPITHELIAL OVARIAN NEOPLASMS

ABSTRACT

INTRODUCTION

Ovarian carcinoma is the 6th most common carcinoma among women in the world and forms 1.7 to 8.7% of female cancers in India.It is the most common cause of gynecological cancer death in women. Surface epithelial ovarian carcinoma accounts for 90 to 95% of ovarian malignancies.

EGFR (Epidermal Growth Factor Receptor)

Among various prognostic indicators, EGFR a 170 Kd glycoprotein maintained its independent prognostic value,and brings about increased DNA synthesis, cell proliferation and differentiation. With the availability of EGFR – inhibitors, selection of patients for EGFR – targeted therapy becomes more important.

VEGF (Vascular Endothelial Growth Factor)

VEGF is a dimeric glycoprotein functioning as a tumour angiogenesis factor.

Bevacizumab – Anti VEGF, antibody shows promise in the treatment of ovarian cancer.

This study is an attempt to determine the expression of the above two markers -EGFR and VEGF in epithelial ovarian neoplasms.

AIMS AND OBJECTIVES:

To study the expression of EGFR (Epidermal Growth Factor Receptor) and VEGF (Vascular Endothelial Growth Factor) in epithelial ovarian neoplasms, which could thence be, used as therapeutic targets in future.

MATERIALS AND METHODS:

30 cases paraffin sections of ovarian specimen diagnosed as borderline and malignant epithelial ovarian neoplasms were subjected to staining with ImmunoHistoChemical markers-EGFR and VEGF.

RESULTS:

Out of 4 borderline ovarian neoplasms, 50% showed positivity for EGFR while 75% of them showed positivity for VEGF.

Among malignancies, 80.76% of them showed EGFR positivity while 84.02% showed VEGF positivity.

CONCLUSION:

With Immunohistochemical analysis, the percentage of EGFR and VEGF expression showed a significant increase in malignant compared to borderline tumours. Even among malignancies, EGFR and VEGF showed a significant

correlation with tumour grade and FIGO stage. High grade and advanced stage tumours showed EGFR and VEGF overexpression compared to low grade and early stage carcinomas.

KEYWORDS:

Surface epithelial ovarian carcinoma, EGFR, VEGF, Immunohistochemical analysis, Bevacizumab.

INTRODUCTION

Ovarian carcinoma is the 6th most common carcinoma among women in the world [1] and it ranks fifth in cancer deaths among women. [2]. Surface epithelial ovarian carcinoma accounts for 90 to 95% of ovarian malignancies [3]

Surface epithelial tumours, statistically the most important group of neoplasms are derived from surface coelomic or germinal epithelium that is continuous with the mesothelium that covers the peritoneal cavity, sharing with it a common origin and many morphological features. The ovarian surface epithelium involved in metaplastic or neoplastic conditions often undergo 'mullerian differentiation' and may produce any of the adult structures formed by the mullerian ducts including tubal, endometrial and endocervical mucosa, singly or in combination [5]. It has also been noted that many of the surface epithelial tumors arise from the invaginated portion of the epithelium that forms surface epithelial glands and cysts [6]. Another proposed origin of some ovarian epithelial tumours (especially serous type) is the epithelium of the tubal fimbriae and fimbriae are the most common sites of early serous carcinoma in women with BRAC mutations [7].

The parameters based on which the surface epithelial ovarian tumors are classified are:

1. Cell histological type: Serous, mucinous, endometroid etc
2. Growth pattern: cystic, solid etc
3. Proportion of fibrous stroma.
4. Degree of atypia and invasiveness: benign, borderline and malignant [6]

A new model divides surface epithelial tumours into 2 major categories: Type 1 and Type 2, based on their clinicopathological features and characteristic molecular genetic changes [8].

Type 1 tumors are slow growing, generally confined to the ovary at the time of diagnosis and developing from well-established precursor lesions [9].

Type 2 tumors are rapidly growing, highly aggressive neoplasms for which well-defined precursor lesions have not been identified. More than 75% of them have TP53 mutations [10].

ROLE OF BIOMARKERS:

Ovarian carcinoma is comparatively asymptomatic in early stage and is aptly called a “silent killer disease”.70% of patients present in stage III and IV underscoring the need for early biomarkers since the survival rates vary significantly with the stage at diagnosis.

TABLE 1: 5 YEAR SURVIVAL RATES FOR EPITHELIAL OVARIAN CANCERS:

STAGE OF THE EPITHELIAL OVARIAN CARCINOMA	5 YEAR SURVIVAL RATE
STAGE I	90%
STAGE II	70%
STAGE III	39%
STAGE IV	17%

"Survival rate for ovarian cancer by stage"- AMERICAN CANCER SOCIETY-retrieved on 29 oct 2014.

BIOMARKERS IN DIAGNOSIS:

The long used CA-125 is raised in only 50% of early stage ovarian cancers [11]. It is also highly non-specific. The need of the hour are other complimentary biomarkers in early diagnosis and prognostication. Two amidst these novel biomarkers are EGFR (Epidermal Growth Factor Receptor) and VEGF (Vascular Endothelial Growth Factor).A multivariate cox analysis regression model showed that high serum VEGF expression in stage I patients is correlated with 8 fold increase in cancer mortality[12].Compared to benign ovarian lesions,early stage ovarian cancer patients showed raised levels of VEGF.Hence when used in combination with CA-125,the sensitivity was increased upto 96% and specificity up to 77%.[13]

BIOMARKERS IN PROGNOSTICATION:

Higher levels of EGFR and VEGF are associated with metastases, development of ascites and poorer prognosis.

BIOMARKERS IN THERAPEUTICS:

It has been predicted that simultaneous inhibition of 2 key tumor dependent growth factor pathways EGFR and VEGF, causes Receptor Tyrosine Kinase (RTK) pathway disruption and consequently tumor growth arrest/inhibition. [13]

EGFR (Epidermal Growth Factor Receptor)

Among various prognostic indicators, EGFR maintained its independent prognostic value. EGFR is a 170KD transmembrane glycoprotein. Ligand binding triggers intrinsic tyrosine kinase activity of the receptor activating numerous cellular responses like increased DNA synthesis, cell proliferation and cell differentiation. With the availability of EGFR inhibitors, selection of patients for EGFR – targeted therapy becomes more important.

VEGF (Vascular Endothelial Growth Factor)

The dimeric glycoprotein VEGF is structurally similar to platelet derived growth factor and may function as a tumour angiogenesis factor. Bevacizumab – anti VEGF, antibody shows promise in the treatment of ovarian cancer. VEGF has been known to have crucial role in neovascular formation in tumors, providing nourishment for the highly metabolic tumor cells and providing access to the host vasculature [12, 13].

Aims and Objectives

AIMS AND OBJECTIVES

1. To study the expression of EGFR (Epidermal Growth Factor Receptor) and VEGF (Vascular Endothelial Growth Factor) in epithelial ovarian neoplasms, which could thence be, used as therapeutic targets in future.

Review of Literature

REVIEW OF LITERATURE

NORMAL ANATOMY AND HISTOLOGY

The ovaries are a pair of female reproductive organs ,lying in the pelvis on either side of the uterus close to lateral pelvic wall, behind broad ligament and anterior to rectum. The mesovarium attaches it to posterior aspect of broad ligament along its anterior margin. The ovarian ligament attaches it to the ipsilateral uterine cornua and infundibulopelvic ligament attaches it to the lateral pelvic wall [14]. Adult ovary has an ovoid shape and measures (3 to 5 cm) x (1.5 to 3 cm) x (0.6-1.5 cm) and weighs 5 to 8 grams during the reproductive period. After menopause, they shrink to one half of this size [15]

LYMPHATICS

The majority of the ovarian lymph vessels drain to large trunks that form a plexus at the hilus and finally drain into Para aortic nodes. Few of them also drain into internal and external iliac, common iliac and inguinal nodes [16]

BLOOD VESSELS

The ovarian artery, a direct branch of the aorta, courses along the infundibulopelvic ligament, anastomoses with the ovarian branch of uterine artery and forms an arcade from which about 10 arterial branches arise and penetrate the ovarian hilus and medulla. These form a plexus at the cortico medullary junction from which the radial cortical arterioles arise [15]. The veins accompany the arteries and finally drain into the ovarian veins. The left ovarian

vein drains into the left renal vein and the right ovarian vein drains into the inferior vena cava [18]

NERVE SUPPLY

Nerve supply to the ovaries is through ovarian, hypogastric and aortic plexuses.

HISTOLOGY

A single layer of cuboidal cells that constitute the germinal epithelium covers the ovarian free surface. The ovarian substance is divisible into cortex and medulla. Immediately deep to the germinal epithelium, the cortex is covered by a condensed connective tissue called the tunica albuginea, which is much thinner and less dense than that of testis. Deep to this, the ovarian stroma is made of slender spindle shaped cells, fine collagen fibres and ground substance. Scattered in this stroma are ovarian follicles at various stages of development – each containing a developing ovum.

The inner medulla consists of connective tissue in which numerous blood vessels are seen.It also contains elastic fibres and some smooth muscle fibres. The ovarian hilus cells are similar to the interstitial cells of the testis.

OOGENESIS

Oogonia are the stem cells from which ova are derived. An oogonium enlarges to form a primary oocyte with diploid number of chromosomes. It undergoes first meiotic division to form two daughter cells with haploid

number of chromosomes. However the cytoplasm is not equally divided and most of it goes to one daughter cell which is large. The second daughter cell with hardly any cytoplasm forms the first polar body. The secondary oocyte undergoes second meiotic division to give rise 2 unequal cells – the larger one is the mature ovum and the smaller one is the second polar body.

FORMATION OF OVARIAN FOLLICLES

The ovum with the surrounding flat stromal cells forms a primordial follicle. These form majority of follicles in the ovary. The flat stromal cells or the follicular cells become columnar and form the primary follicle. The follicular cells proliferate to form several layers of granulosa cells. The homogenous membrane – the 'zona pellucida' appears between the follicular cells and the developing ovum. This is a 'secondary follicle'. A follicular cavity – the antrum appears, filled with a fluid – the liquor folliculi. The oocyte lies eccentrically in the follicle surrounded by some granulosa cells, the cumulus oophorus. As the follicle expands – the stromal cells surrounding the granulosa become condensed to form a covering called "theca interna" outside which some fibrous tissue becomes condensed to form another covering for the follicle – "the theca externa". The first meiotic division is completed just before ovulation to form the secondary oocyte. Follicular antrum enlarges markedly. The follicle reaches the size of 1.5 cm to 2.5 cm and bulges under the ovarian surface as the mature 'Graafian follicle'

CORPUS LUTEUM

When the graafian follicle ruptures, it collapses and becomes folded and fills with blood. The granulous cells are enlarged with abundant pale cytoplasm and round nuclei, abundant smooth endoplasmic reticulum and mitochondria and numerous lipid droplets giving a yellow tinge and hence the name“corpus luteum” which secretes progesterone.

EMBRYOLOGY

OVARY

Formed essentially from the gonadal ridge.

OVA

In early embryonic phase, the primordial germ cells are formed from the dorsal endoderm of the yolk sac and migrate along the hindgut to the gonadal ridge [24].

DESCENT OF THE OVARIES

From the lumbar region, ovaries descend to the pelvic cavity by the pull of gubernaculum ovarii which stretches from ovary to the skin of labium majus. Their descent is arrested at the pelvis by the developing uterus and the broad ligament.

FUNCTIONS OF THE OVARY

1. Gamete production associated with periodical release of ova.
2. Endocrine functions – Ovaries secrete estrogen, progesterone and small amount of testosterone [21, 22]

TUMOURS

WHO classification of Ovarian tumours given under Annexure 1

SURFACE EPITHELIAL TUMORS

Form two-thirds of all ovarian neoplasms [23]. They are further classified according to the following parameters

a. Histological Cell type – serous, mucinous, endometrioid etc
b. Growth pattern – cystic, solid etc
c. Proportion of fibrous stroma.
d. Degree of atypia and invasiness – benign, borderline and malignant

EPIDEMIOLOGY

In western countries, ovarian carcinoma is the most common cause of gynaecological cancer death. It constitutes 4% of total carcinomas in women [93]. The approximate risk of American women developing ovarian carcinoma in their lifetime is 1.4%. Generally, we can say that the disease is seen more commonly in industrialized western countries because of their low parity, an important exception being Japan, because though the parity is lower, they have relatively lower incidence of ovarian carcinoma.On the other hand, Scandinavia shows one of the highest annual incidence rates of more than 16 per 1 lakh females [94]

The incidence of ovarian carcinoma in India, ranged from 1.7% to 8.7% of all female cancers as per the data gathered from urban and rural registries,

working under the network of National Cancer Registry Programme (NCRP) of ICMR (Indian Council of Medical Research) [107]. The total number of new cancer patients in India is well on the rise partly due to the increase in population and also due to the relative rise in the proportion of elderly population due to improved life expectancy. Ovarian carcinoma ranks third/fourth among the cancers occurring in women in India. In the national cancer registries from Ahmedabad / Bengaluru and Chennai, an increase in the mean annual percentage change was noted in age group of 55-64 years [108]. Lifestyle changes towards industrialization and urbanization in India , especially rise in age at marriage, delay in age at first birth, reduced parity, increase in incidence of obesity, diabetes, hypertension, cancer corpus uterus-, diet rich in saturated/animal fats have all contributed to the increased incidence of ovarian carcinoma in India. Maximum increase over the last 10 year period was observed in Nagpur with the mean annual percentage increase of 2.4%. Some of this increase is also attributed to improved certification and registration of the disease in the recent years.

In the recent years, one significant change noted is that there is a relative fall in the incidence of ovarian carcinoma, as tubal carcinoma and peritoneal carcinomas have started showing an increasing trend [95]

Migration studies show that, the rate of ovarian carcinoma is determined by the immigration place rather than the emigration place - indicating a significant environmental component in the risk of ovarian carcinoma.

The incidence of ovarian carcinoma also shows a distinct variation according to the ethnicity. White women have increased incidence compared to African-American and Asians. Asian women have a 48% lower death rate compared to that of white women. Jews have eight times increased risk of developing ovarian carcinoma compared to non-Jewish women because 1 in 40 of them have a BRCA1 mutation [96]

ETIOLOGY AND RISK FACTORS

1. **Age:** Risk increases with age. Mean age in India is 40-59 years. The average age of women affected in hereditary syndromes (like Lynch syndrome) is much lower than others.
2. **Reproductive factors:** Early menarche and late menopause are significant risk factors. Increase in number of pregnancies and consistent oral contraceptive pill usage are proved to be protective against ovarian carcinoma. Increase in number of pregnancies appear to be relatively more protective against endometroid and clear cell carcinoma subtypes.
3. **Ovulation and Hormonal factors:** "Incessant ovulation" predisposes to malignant transformation of the actively proliferating surface epithelium. The occurrence of ovarian carcinoma is directly linked to the total duration of reproductive years without interruption by pregnancies (or) oral contraceptive pill usage. Recent studies support the fact that consistent oral contraceptive pill usage reduces the risk of ovarian cancer by 50%. The protective effect becomes stronger with longer

duration of usage. Another theory says that increased levels of circulating gonadotrophins increases the chance of incidence of ovarian cancer either directly (or) by increasing the circulating levels of oestrogen. Another theory proposes that the levels of androgens are also important in the causation of ovarian carcinoma.

4. **Inflammation:** High grade serous carcinomas are associated with chronic salpingitis in 53% of cases [24]
5. **Others:** Include Body Mass Index, diet, talc, smoking, ionizing radiation, surface epithelial dysplasia, surface epithelial inclusions, endometriosis, serous tubal intraepithelial carcinoma. Other important protective factors include hysterectomy, fallopian tube ligation, and bilateral salpingo – oophorectomy, -the protective mechanism being prevention of retrograde passage of endometrial tissue and, endometriosis. Hence the incidence of clear cell carcinoma varies inversely with tubal ligation [97]
6. **Genetic Factors:** At least 10% of ovarian carcinomas arise in the setting of highly penetrant, autosomal dominant genetic predisposition. These include BRCA1 and BRCA2, HNPCC (Hereditary Nonpolyposis Colorectal Cancer Syndrome), Lynch syndrome [25]

CLINICAL FEATURES

The patient often presents with vague, nonspecific symptoms like bloating, abdominal distension, dyspepsia, lower abdominal pain, loss of appetite and loss of weight, nausea, vomiting, increased frequency and urgency to urinate [26].

PROBABLE HISTOPATHOLOGICAL PRECURSOR LESIONS

1. Surface epithelial Dysplasia

Recent investigations indicate that subtle nuclear changes were seen in ovaries removed prophylactically from high risk women compared to normal controls [98].

2. Surface epithelial inclusions

Several studies have shown that ovaries of prophylactic oophorectomy specimens from high risk women showed invaginations of cortical epithelium (clefts) and papillomatosis more commonly than in controls.

3. Endometriosis

The best studied and most easily recognized precursor lesion is “endometriosis”. Endometriosis is a common lesion found in about 10% of reproductive age women. A series of studies support the fact that endometriosis was at least as common as serous cystadenoma and hence would be the most common benign ovarian lesion.

Extensive studies also show that the incidence of carcinoma in a known case of endometriosis is just 0.3 to 3% [99]. In a Sweden based study of more than 20,000 hospitalized women with endometriosis, a 11.4 year follow-up showed that the relative risk of carcinoma in ovarian endometriosis is 1.9. The mean age of occurrence of carcinoma in these cases was 51 – showing that the incidence of carcinoma in endometriosis occurs in a relatively younger age group.

4. Benign and atypical proliferating neoplasms

Molecular analysis studies, strongly suggest that borderline tumours are forerunner lesions of low grade serous, endometroid and mucinous carcinomas.

5. Serous Tubal Intraepithelial carcinoma (STIC) and p53 signature

There is a recent proposal that fallopian tubal fimbriae are the origin of some of serous carcinomas. Serous tubal Intraepithelial Carcinomas (STICs) have been found to be associated with greater proportion of high grade serous carcinomas [100]. These STIC lesions harbour TP53 mutations. Though they are cytologically malignant lesions, they are confined to the tubal epithelium. A minimum of 12, p53 positive, fallopian tubal secretory epithelial cells define a case of “p53 signature”. This p53 signature being a candidate for STIC precursor.

The junctions between different types of epithelium have long been known as “Hot Spots” for origin of carcinomas. Similarly, Tubal-Peritoneal

junction (TPJ) or the meeting zone of peritoneum with fimbrial epithelium has been evaluated as the source of serous carcinomas.

PREVENTION

A study involving more than 80,000 women has shown that there exists an inverse relation between caffeine intake and ovarian carcinoma risk. Smoking has been found to increase the risk of mucinous carcinoma.Avoidance of smoking and all other possible risk factors may play a role in prevention, to a certain extent.

STAGE AND PATTERN OF SPREAD

Grading and FIGO staging of ovarian carcinoma has been given in the annexure.

FIGO stage appears to be the most powerful predictor of outcome in ovarian carcinoma compared to most other prognostic factors. Histological type of ovarian carcinoma determines the stage of presentation. Most of the mucinous subtypes presented in stage I while only about 3% of serous carcinomas presented in stage I. A series of numerous studies show that only 14% of ovarian carcinomas presented in stage I. Most common presentation of carcinoma ovary is in stage III and 84% of stage III carcinomas were stage III C, involving spread to the abdominal (or) extra pelvic peritoneum [101]. Two thirds of cases of ovarian carcinoma show presence of ascites.

The incidence of lymph node metastasis varies with the stage. Stage I tumours show lymph node metastasis in about 9% of cases, stage II – 36%, stage III 55%, and stage IV tumours show lymph node metastasis in about 88% of cases. Volume of residual disease forms an important prognostic factor for stage III and IV carcinomas.

Stage IV tumours include those showing distant metastasis and includes patients with liver parenchymal metastasis and extra abdominal metastasis. Lung and pleural metastasis are seen in up to 45% of patients with ovarian carcinoma, one of the most common causes of death among ovarian carcinoma patients being respiratory failure. Metastasis to liver- seen in up to 50% of ovarian carcinoma patients at autopsy. The average period of survival of patients with liver metastasis is about 1 year. Skin and subcutis of periumblical region have been the most frequent site of anterior abdominal wall metastasis. Only 0.1% of patients show brain metastasis at presentation. 1-2% of patients develop bone metastasis during the disease course.

SEROUS TUMORS

Constitute one fourth of all ovarian tumors of which 30% to 50% are bilateral, 75% are benign or borderline while 25% of these are malignant [27]. The serous cystadenocarcinomas are the most common of all malignant ovarian tumors. Common age group affected is between 20 to 50 years [22]. Grossly they are solid and cystic with often papillary excrescences, areas of haemorrhage and necrosis. Microscopically the cystic areas are formed by tall

columnar cells and filled with clear serous fluid. Borderline tumors may have cellular atypia and stratification but there is no evidence of invasion. 'Psammoma bodies' if present are pathognomonic of papillary serous cystadenocarcinomas. Micropapillary serous carcinomas are characterized by a pattern of highly complex micropapillae arising from large bulbous papillary structures and are characterized by higher rates of recurrence. Immunohistochemically, they are typically, CK7, WT1 and CA125 positive. The 5 year survival rate of borderline and malignant tumors are 90% and 25% respectively.

MUCINOUS TUMORS

These are less common and are bilateral in only 10-20% of cases [29]. Microscopically divided into 2 major types the intestinal type wherein the epithelial lining shows 'picket fence' appearance, goblet cells, paneth cells etc [30]. The endocervical or the mullerian type shows endocervical type lining epithelium [31]. Stromal invasion differentiates borderline from malignant tumors. 10 year survival rate for borderline and malignant mucinous tumors are 90% and 65% respectively. IHC – positive for CDX2, CEA, CK20, CA125 etc.[32]

ENDOMETRIOID TUMORS

Comprise 10-25% of all primary ovarian carcinomas. Endometriotic findings noted in 10 to 20% of cases [33]. 15 to 30% of cases show concomitant endometrial hyperplasia or carcinoma. Microscopically made of endometrial tubular glands. 40% of these tumors are bilateral tumors. Borderline tumors have a complex branching pattern without stromal invasion. 5 year survival rate for tumors confined to the ovary is 75%.

CLEAR CELL TUMORS

Microscopically grow in tubulocystic, papillary pattern and solid sheets [36]. The tumor cells are large with clear cytoplasm and nuclear hobnailing. [35]. IHC – CK7, CA125 positive and negative for CK20. Bilateral in less than 10% of cases. They are aggressive tumors showing less response to chemotherapy than other ovarian carcinomas. They have a very high association with pelvic endometriosis.

BRENNER TUMOR AND TRANSITIONAL CELL CARCINOMA

Constitute 1-2% of all ovarian neoplasms [36]. Some are accompanied by signs of hyperestrinism. Microscopically consist of nests of urothelium-like cells surrounded by abundant fibroblastic stroma. The nuclei may exhibit longitudinal grooves. Transitional cell carcinomas are those without the accompanying benign component. They all originate from surface ovarian epithelium through the process of metaplasia [37]

SQUAMOUS CELL TUMORS:

Primary squamous cell carcinoma ovary is exceedingly rare.They usually occur in ovaries as part of mature teratoma with malignant transformation of the squamous elements,or as metastasis from non-ovarian sources[119].Squamous elements may sometimes occur rarely as part of a metaplastic process in an endometrioid carcinoma ovary.Squamous cell carcinoma ovary is an aggressive ovarian tumor.CA-125 is either normal or is only mildly elevated in case of primary squamous cell carcinoma ovary.Presents radiologically as a heterogeneously echoic solid and cystic mass.Microscopically identified with obvious invasion into the stroma.Keratin formation and intercellular bridges seen in well-differentiated forms.Treatment is surgery with adjuvant chemotherapy,but the prognosis is poor.[120].

MIXED EPITHELIAL TUMORS:

As per WHO classification,mixed epithelial tumors are those in which the minor component is easily recognizable and should constitute atleast 10% of the tumor on microscopic examination.Mixed epithelial tumors ovary constitute <4% of all epithelial ovarian neoplasms.Serous-endometrioid,serous-transitional,endometrioid-clear cell carcinoma types are the most frequent combinations seen[116].The dominant cell type determines the biological behaviour of the tumor.These tumors pose a diagnostic dilemma.Hence study of multiple sections of a tumor is recommended to exclude a mixed epithelial tumor.Mixed serous-endometrioid and serous-clear cell carcinomas usually are

a representation of high grade serous carcinoma with areas that mimic endometrioid and clear cell carcinoma.Clear cell carcinoma and endometrioid carcinoma usually arise in the setting of endometriosis and hence may occur in combination.In case of endometrioid and undifferentiated carcinoma occurring together,we need to exclude the possibility of dedifferentiated endometrioid carcinoma rather than a mixed epithelial tumor.[118].

MALIGNANT MIXED MULLERIAN TUMORS

More common in the uterus than in the ovary. The carcinomatous component maybe of serous, endometrioid, squamous or clear cells. The most common sarcomatous component is chondrosarcoma. Prognosis is extremely poor.

ADENOSQUAMOUS CARCINOMA AND OTHER EPITHELIAL TUMORS

Primary adenosquamous carcinoma of ovary is an extremely rare malignancy occurring in <1% of all malignant ovarian tumors [38]. Microscopically cells are arranged in sheets, glandular and focal papillary pattern. The cells show high grade pleomorphic vasicular nuclei. Also seen are cells showing malignant squamous differentiation with keratin pearls [39.]Because of the rarity,the optimal management of primary adenosquamous carcinoma ovary is unclear.Expression and immunohistochemical staining intensity of EGFR and VEGF has been noted to be stronger and more prevalent in squamous cell carcinoma compared to adenosquamous carcinoma.[115].

UNDIFFERENTIATED CARCINOMA:

These are a diagnosis of exclusion when the characteristic or diagnostic histological differentiating feature is absent.Before diagnosing this we need to exclude metastatic carcinomas and other non-epithelial neoplasms.It is sometimes hard to elicit the epithelial differentiation of these tumors even with immunohistochemistry.

PROGNOSTIC FACTORS

1. Age – Younger patients show better outcome
2. BRCA1 mutations and family history
3. Tumour stage and grade
4. Ascites – Unfavourable prognostic sign.
5. Psammoma bodies indicate better prognosis
6. DNA ploidy – aneuploid tumors show higher grade.
7. CA-125 levels.
8. P53 – overexpression associated with poor prognosis
9. Tumor angiogenesis
10. Histological type.
11. Intratumoral T cells
12. Other markers including EGFR, VEGF etc are all associated with aggressive behaviour

.

TREATMENT

Depends on tumour stage and grade. Surgery is the initial treatment of choice followed by chemotherapy with taxane or a platinum compound. Even in late stages, debulking surgery reduces the tumour burden. Primary cytoreductive surgery (at presentation) and secondary cytoreductive surgery (on recurrence) prolong the survival and progression free interval.

Patients with advanced stage (FIGO III and IV) disease benefit from chemotherapy utilising platinum based compounds with or without a taxane. Platinum-based compounds have been shown to prevent extra abdominal metastasis. Paclitaxel has been found to be useful in many patients with platinum resistance.

Newer Modalities: Gefitinib – an EGFR (Epidermal Growth Factor Receptor) inhibitor has shown promise in the treatment of ovarian carcinoma since EGFR amplification is noted in about 20% to 80% of ovarian carcinomas. Bevacizumab – a VEGF (Vascular Endothelial Growth Factor) inhibitor is another drug showing promise in the ovarian carcinoma management [102]

IMMUNOHISTOCHEMISTRY

IHC refers to the process of detecting antigens in cells, by using specific antibodies [40, 41]. The procedure was first initialized by Dr. Albert Coons in 1941. A number of ways are present to visualize the antigen-antibody interaction [42]

Some of the methods, are where the antibody is conjugated to an enzyme, like peroxidase which catalyzes a color producing reaction [43, 44]

Sometimes the antibody is tagged to a fluorophane like fluoroscein or rhodamine [45, 46, 47]

STEPS IN IMMUNOHISTO CHEMISTRY

1. Tissue processing and antigen or epitope retrieval.
    a. 10% neutral buffered formalin is the preferred fixative.
    b. These fixatives cause certain reversible changes in tertiary and quarternary structure of proteins [48, 49]
    c. Formalin fixed paraffin embedded tissue sections are cut 3 to 4 microns thick and mounted on glass slides.
    d. Trypsin or protease enzyme digestion or
    e. Heating in buffered solutions example- citrate or EDTA buffer in either a microwave oven or pressure cooker" retrieves "or "unmasks "the antigens that have been altered by formalin fixation [50, 51, 52].
2. Antigen – antibody interaction

    Either the direct or indirect method can be used.
3. Visualizing with detection systems

    Antibody molecules can be labelled with either fluorescent compounds or active enzymes. Horse radish peroxidase enzyme is

commonly used. The chromogens added thereafter are oxidized by horseradish peroxidase enzyme – giving a resultant brown/red colored IHC staining [53, 54, 55]

EGFR

The epidermal Growth Factor Receptor structure- wise has an extracellular ligand binding domain, a transmembrane spanning region and an intracellular kinase containing domain [56, 57]. Activation of EGFR causes transmission of signals via intracellular MAPKS – Mitogen Activated Protein Kinases and protein kinase B causing a multitude of cellular responses like proliferation, cell motility and survival [58, 59, 60]. The EGFR gene is located on chromosome 7p12 [61, 62]. It is overexpressed in 9-62% of human ovarian cancers [63, 64]. Increased expression is linked with higher tumor grade, high proliferation index and poor patient outcome [65].

The normal epithelial lining of ovary has got weak EGFR expression. Epithelial ovarian carcinomas show overexpression of EGFR in 4-100% of cases [103].

Therapeutic implications of EGFR: Small molecule Tyrosine Kinase Inhibitors (TKIs) and monoclonal antibodies have been used currently in blocking EGFR activity. Erlotinib is the most common TKI. It is orally active, potent and also selectively inhibits EGFR Tyrosine Kinase. It binds reversibly to the ATP-binding site of EGFR and also inhibits autophosphorylation by

EGFR tyrosine kinase. This causes blockade of all subsequent EGFR signal transduction pathways producing cell cycle arrest. Next to EGFR TKIs, anti EGFR monoclonal antibodies like Cetuximab are the ones to be studied most extensively. [104]

VEGF

A specific mitogen for vascular endothelial cells – the VEGF is a heparin binding dimeric polypeptide [66, 67]. For VEGF epithelial expression, about 5% of benign cystadenomas, 30% of borderline tumors and 80% of epithelial carcinomas showed positive staining [68, 69]. The expression of VEGF is increased in response to hypoxia, oncogenes and numerous cytokines. VEGF causes endothelial cell proliferation, cell migration and apoptosis inhibition. It also regulates angiogenesis [70].

Though there are several angiogenic factors, VEGF (Vascular Endometrial Growth Factor) happens to be the single most robust molecule in the process of angiogenesis. There is a direct correlation of VEGF with intratumoral microvessel density. It heralds a poor prognosis in cancer patients. VEGF inhibition has been shown to reduce the tumour vessel density and tumour growth.

VEGF-A gene is located on chromosome – 6p12. Hyoxia Responsive Elements (HREs) are present in this gene. Hence Hypoxic conditions including Tumour hypoxia upregulates VEGF expression. VEGF induced angiogenesis is

responsible for malignant ascites production and eventual disease progression [105]. Even for patients with early stage disease, elevated VEGF levels were associated with significant risk of recurrence.

VEGF targeting therapies

Two primary strategies to inhibit the VEGF pathway are

1. Inhibiting binding of VEGF ligand with antibodies
2. Inhibiting binding of VEGF with tyrosine kinase inhibitors

Bevacizumab

It is a 149 KDa recombinant humanized monoclonal anti-VEGF antibody. Two pivotal phase II trials have evaluated the efficacy of bevacizumab for the treatment of recurrent epithelial ovarian, peritoneal or tubal carcinoma. These trials showed a tremendous response rate of 20 – 60% , in achieving stable phase [106].

Other VEGF receptor Tyrosine Kinase inhibitors that have been evaluated are Ramncirumab, Cediranib, Semoxanib, Sunitinib, Sorafenib, Vatalanib, Vandetanib, Intedanib, pazopanib etc…

Hence we find that VEGF is an attractive target for therapeutics and drug research in ovarian carcinoma

Materials and Methods

MATERIALS AND METHODS

This study is a retrospective one conducted at Institute of Social Obstetrics and Govt Kasturba Gandhi Hospital for Women and Children, Madras Medical College, Chennai for a 3 year study period from 2013 to 2015. Out of the total 9313 cases of histopathological specimens received, 171 were ovarian neoplasms,out of which 92 were surface epithelial ovarian neoplasms.Out of these 92 suface epithelial ovarian neoplasms,62 were benign, 4 were borderline and 26 were malignant.

DATA COLLECTION

Case details especially age, complaints, procedure done, grade and stage of tumors were obtained from pathology registers. Hematoxylin and Eosin sections of the paraffin tissue blocks were reviewed. Out of the 92 surface epithelial ovarian neoplasms, 26 ovarian malignancies and 4 borderline tumors selected and their corresponding paraffin tissue blocks obtained for immunohistochemical analysis of EGFR and VEGF.

TABLE 2: PROCEDURE OF IMMUNOHISTOCHEMISTRY

Antigen	Vendor	species (clone)	Positive Control
EGFR	PathnSitu	Rabbit monoclonal	Squamous cell Carcinoma
VEGF	PathnSitu	Mouse Monoclonal	Kidney

1. 4 micron thick sections were cut from formalin fixed paraffin embedded tissue blocks and transferred onto gelatin –chrome-alum coated glass slides
2. The glass slides were kept in an incubator at 58 degree Celsius overnight.
3. Deparaffinisation in xylene for 15 minutes x 2 changes
4. Dehydration with absolute alcohol for 5 minutes x 2 changes
5. Washing of sections done in tap water for 10 minutes
6. Then in distilled water for 5 minutes
7. Retrieval of antigen done with microwave oven with sections immersed in Tris EDTA buffer for 20 minutes

a. 800 watts – 5 minutes

b. 600 watts – 10 minutes

c. 400 watts – 5 minutes

8. Cool the slides to room temperature and then washed with distilled water for 10 minutes.
9. Then washed in phosphate buffer for 5 minutes x 2 changes
10. Application of peroxidase block over the sections for 10 minutes
11. Slides washed with phosphate buffer for 5 minutes.
12. Appropriate primary antibody was applied over the sections and incubated for half an hour.
13. After washing with wash buffer, polyexcel target binder reagent applied for 15 minutes.
14. Slides were washed with 2 changes of buffer for 2 minutes.

15. Sections were covered with HRP micropolymer for 15 minutes
16. Washed with phosphate buffer for 2 minutes
17. 1 drop of DAB chromogen (prepared by diluting 1 drop of DAB chromogen to 1 mL of DAB buffer) was applied for 2-5 minutes
18. Counterstaining was done with hematoxylin, washed in running tap water, air dried, cleared with xylene and mounted.

INTERPRETATION AND SCORING

The IHC slides were analysed for the presence of the reaction, cellular localization of the staining – EGFR shows membrane and/or cytoplasmic staining. VEGF also shows cytoplasm and /or membrane staining. Percentage of tumor cells taking up the stain and the intensity with which they stain were also analysed.

STATISTICAL ANALYSIS

Performed with package for social science software version 11.5. The expression of EGFR, VEGF were correlated and studied using student t-test and chi square test.

Observation and Results

OBSERVATION AND RESULTS

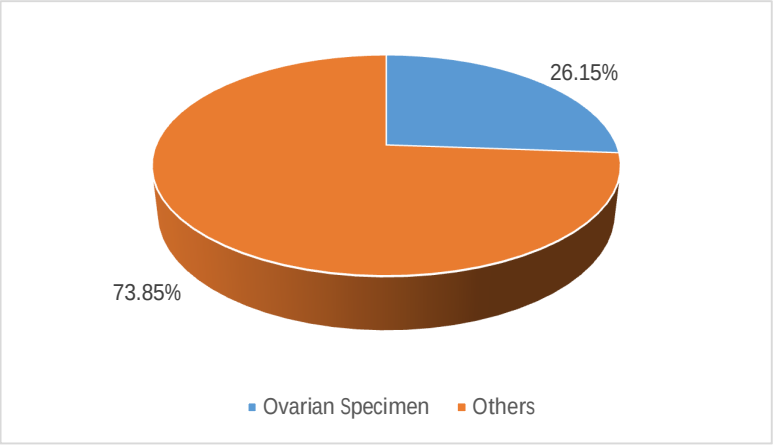
In the 36-month study performed from June 2012 to June 2015, total of 9313 specimen were received at the Department of Pathology, Institute of social obstetrics and Govt. Kasturba Gandhi Hospital for women and children for histopathological examination. Out of the total 9313 cases, Ovarian specimen were 2435, of which, 171 were neoplastic, 1418 were normal and 846 were non-neoplastic.

Thus ovarian specimen received constituted (26.15%) of the total histopathological specimen (Table 3, Chart 1)

TABLE 3: FREQUENCY OF OVARIAN SPECIMEN AMONG TOTAL HISTOPATHOLOGICAL SPECIMEN

	Count	Percentage
Ovarian Specimen	2435	26.15%
Others	6878	73.85%

CHART 1: FREQUENCY OF OVARIAN NEOPLASMS AMONG TOTAL HISTOPATHOLOGICAL SPECIMEN:

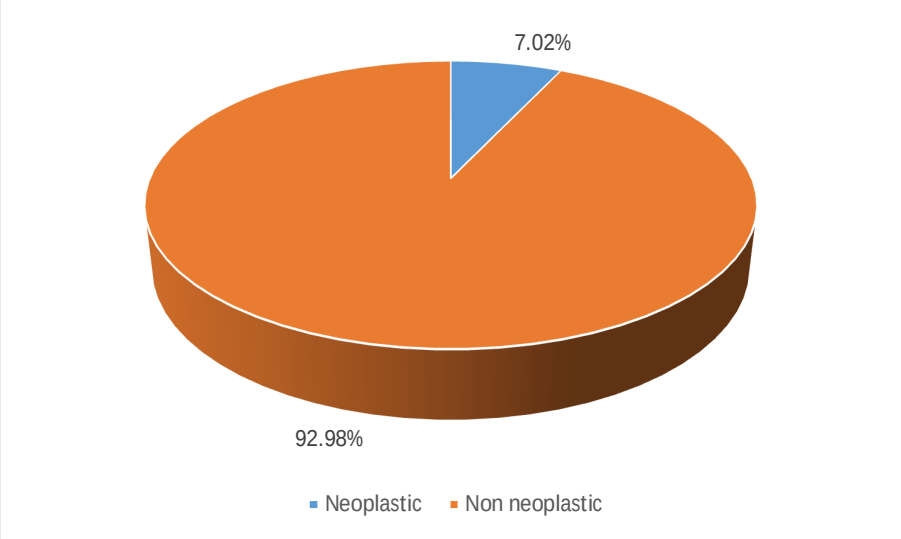


- Amidst ovarian lesions, 846 were non-neoplastic and 171 were neoplastic (Table 4, Chart 2).

TABLE 4: FREQUENCY OF NONNEOPLASTIC AND NEOPLASTIC LESIONS OVARY

	Count	Percentage
Neoplastic	171	7.02%
Non neoplastic	846	92.98%

CHART 2: FREQUENCY OF NON-NEOPLASTIC AND NEOPLASTIC LESIONS OVARY

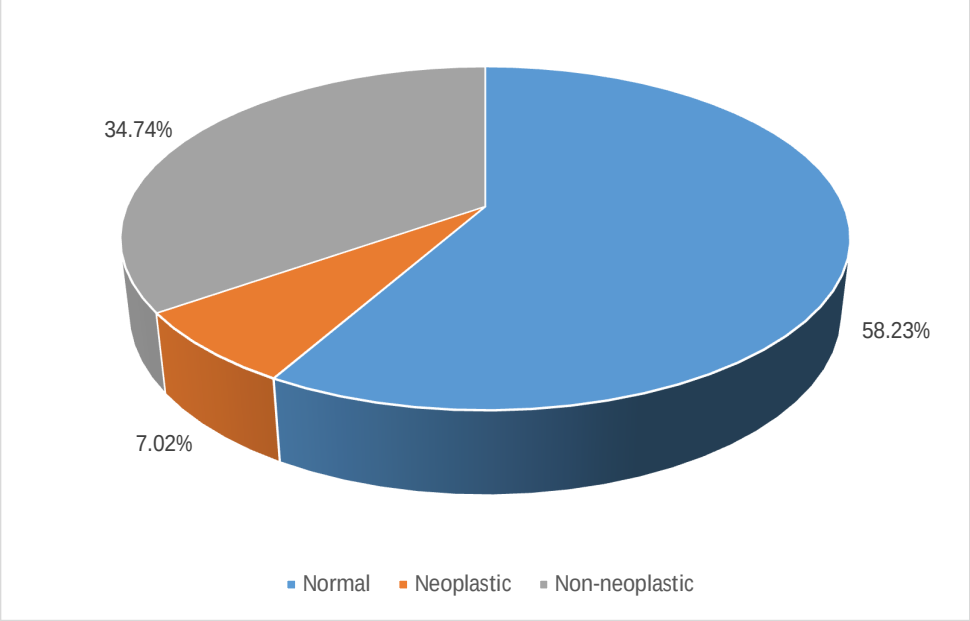


- Hence amidst total ovarian specimen of 2435, normal ovaries were 1418 constituting 58.23%, non-neoplastic ovaries were 846 constituting 34.74% and neoplastic ovaries were 171 constituting 7.02%(Table 5,chart 3).

TABLE 5:FREQUENCY OF NORMAL, NEOPLASTIC AND NON-NEOPLASTIC OVARIES

	Count	Percentage
Normal	1418	58.23%
Neoplastic	171	7.02%
Non neoplastic	846	34.74%

CHART 3: FREQUENCY OF NORMAL, NEOPLASTIC AND NON-NEOPLASTIC OVARIES

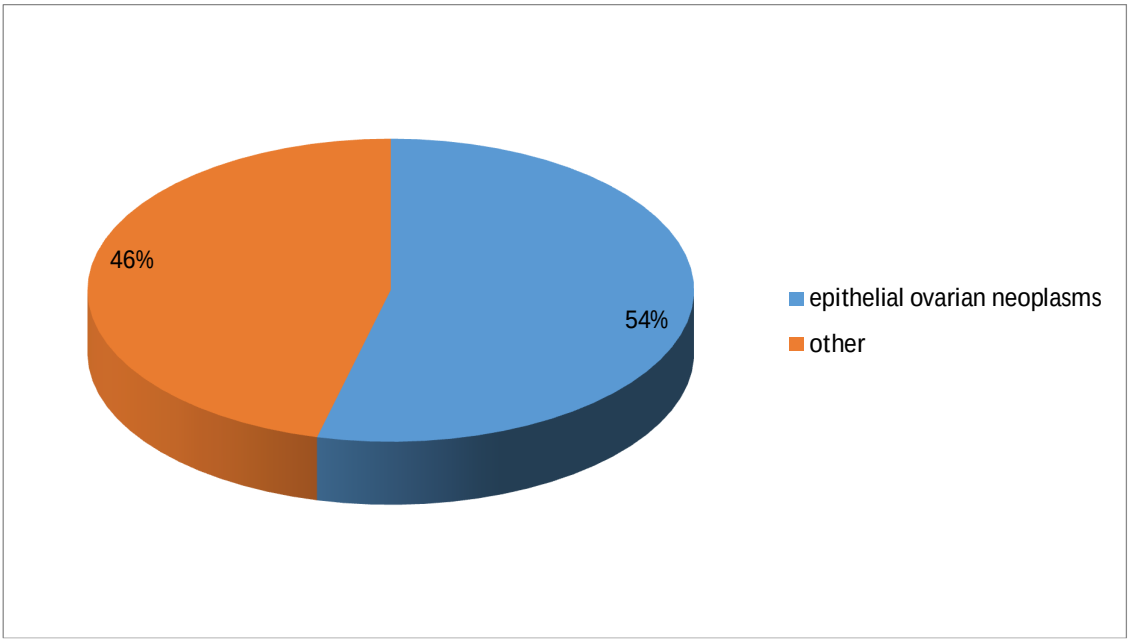


- Amidst 171 ovarian neoplasms, 92 were surface epithelial ovarian neoplasms that constituted 53.801% of total ovarian neoplasms, and hence topped the list of total ovarian neoplasms and were statistically significant (Table 6, Chart 4).

TABLE 6: FREQUENCY OF EPITHELIAL OVARIAN NEOPLASMS

	Count	Percentage
Epithelial-Ovarian Neoplasms	92	53.8%
Others	79	46.2%

CHART 4: FREQUENCY OF EPITHELIAL OVARIAN NEOPLASMS

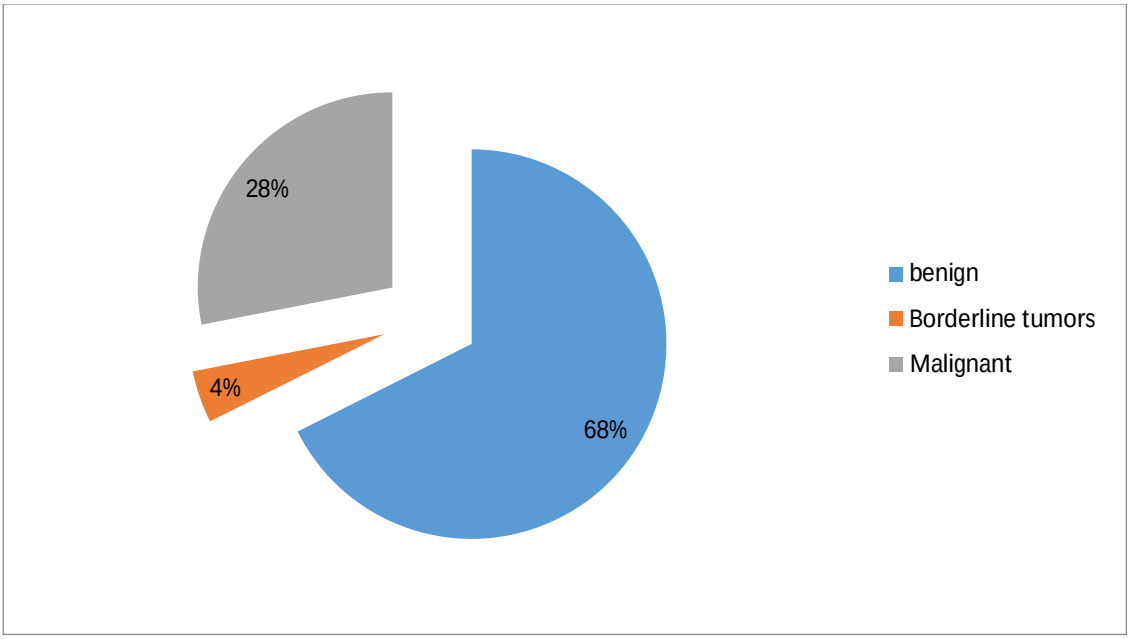


- Amidst 92 surface epithelial ovarian neoplasms, 62 were benign, 4 were borderline tumours and 26 were malignant (Table 7, Chart 5).

TABLE 7: FREQUENCY OF BENIGN ,BORDERLINE AND MALIGNANT EPITHELIAL OVARIAN NEOPLASMS.

	Count	Percentage
Benign	62	68%
Borderline	4	4%
Malignant	26	28%

CHART 5: FREQUENCY OF BENIGN,BORDERLINE AND MALIGNANT EPITHELIAL OVARIAN NEOPLASMS.

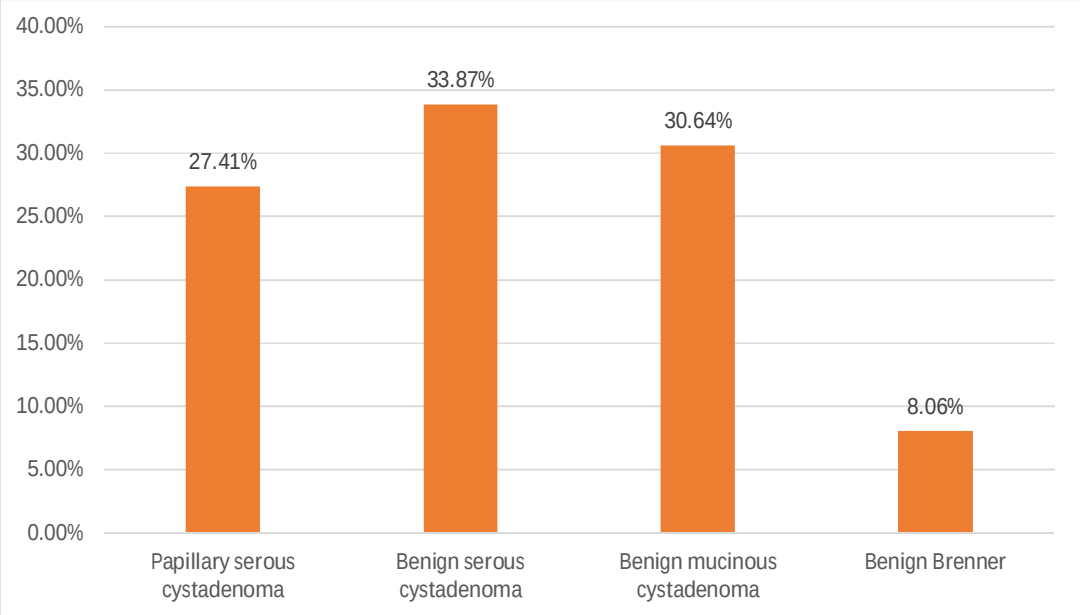


- Amidst the 62 benign ovarian surface epithelial tumors, the frequency of distribution of different histopathological types were-(Table 8, Chart 6)

TABLE 8: HISTOMORPHOLOGICAL DISTRIBUTION OF BENIGN SURFACE EPITHELIAL OVARIAN NEOPLASMS:

	Count	Percentage
Papillary serous cystadenoma	17	27.41%
Benign serous cystadenoma	21	33.87%
Benign mucinous cystadenoma	19	30.64%
Benign Brenner	5	8.06%

CHART 6: HISTOMORPHOLOGICAL DISTRIBUTION OF BENIGN SURFACE EPITHELIAL OVARIAN NEOPLASMS:



- Amidst the 4 borderline tumours, 2 were atypical proliferating serous tumours (50%), 2 were atypical proliferating mucinous tumours (50%) (Table 9)

TABLE 9: HISTOMORPHOLOGICAL DISTRIBUTION OF BORDERLINE TUMORS.

	Count	Percentage
Atypical proliferating serous tumour	2	50%
Atypical proliferating mucinous tumours	2	50%

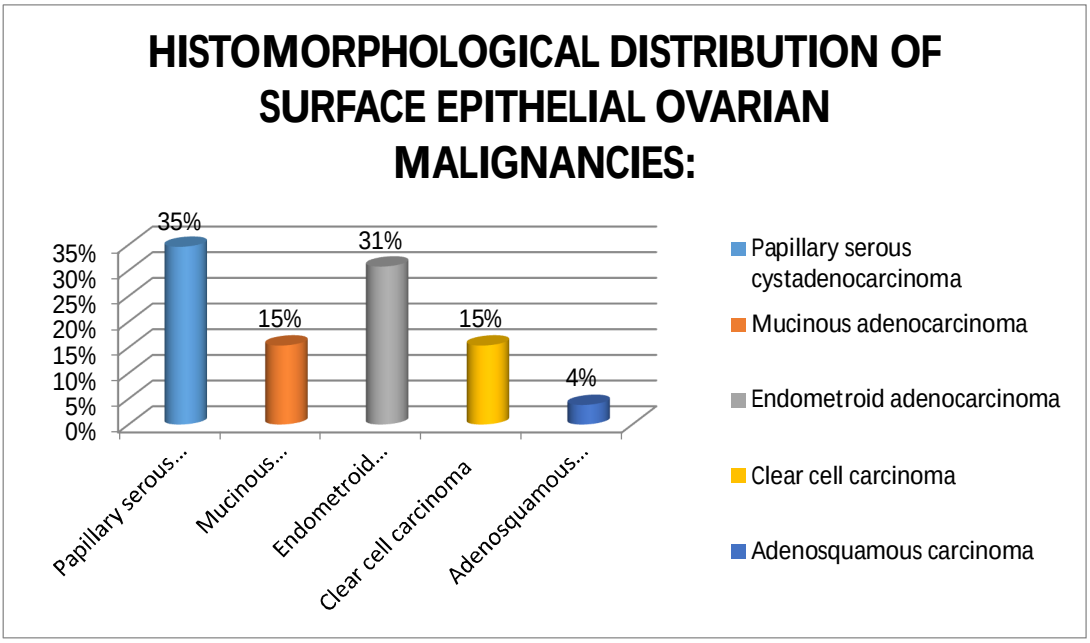
Hence Benign serous tumours top the list constituting about 61.3% of total benign epithelial ovarian neoplasms, closely followed by benign mucinous cystadenomas that constituted about 30.64% of total benign epithelial ovarian neoplasms.

Amidst the 26 surface epithelial ovarian malignancies, the different histopathological types were as in (Table 10, Chart 7).

TABLE 10: HISTOMORPHOLOGICAL DISTRIBUTION OF SURFACE EPITHELIAL OVARIAN MALIGNANCIES

	Count	Percentage
Papillary serous cystadenocarcinoma	9	34.61%
Mucinous adenocarcinoma	4	15.38%
Endometroid adenocarcinoma	8	30.76%
Clear cell carcinoma	4	15.38%
Adenosquamous carcinoma	1	3.81%

CHART 7: HISTOMORPHOLOGICAL DISTRIBUTION OF SURFACE EPITHELIAL OVARIAN MALIGNANCIES



Benign epithelial ovarian neoplasms had a peak incidence at age group of 31 – 40 years that constitutes about 40.24% followed by the age group of 41 – 50 years that formed about 24.38%. Mean age is about 33.33 years(Table 11,Chart 8).

TABLE 11: AGE WISE DISTRIBUTION OF BENIGN EPITHELIAL OVARIAN NEOPLASMS

Age group	Number of cases	Percentage
21-30 years	5	10.97%
31-40 years	29	40.24%
41-50 years	16	24.38%
51-60 years	9	15.86%
>60 years	3	8.53%
Total cases	62	100%

CHART 8: AGE WISE DISTRIBUTION OF BENIGN EPITHELIAL OVARIAN NEOPLASMS

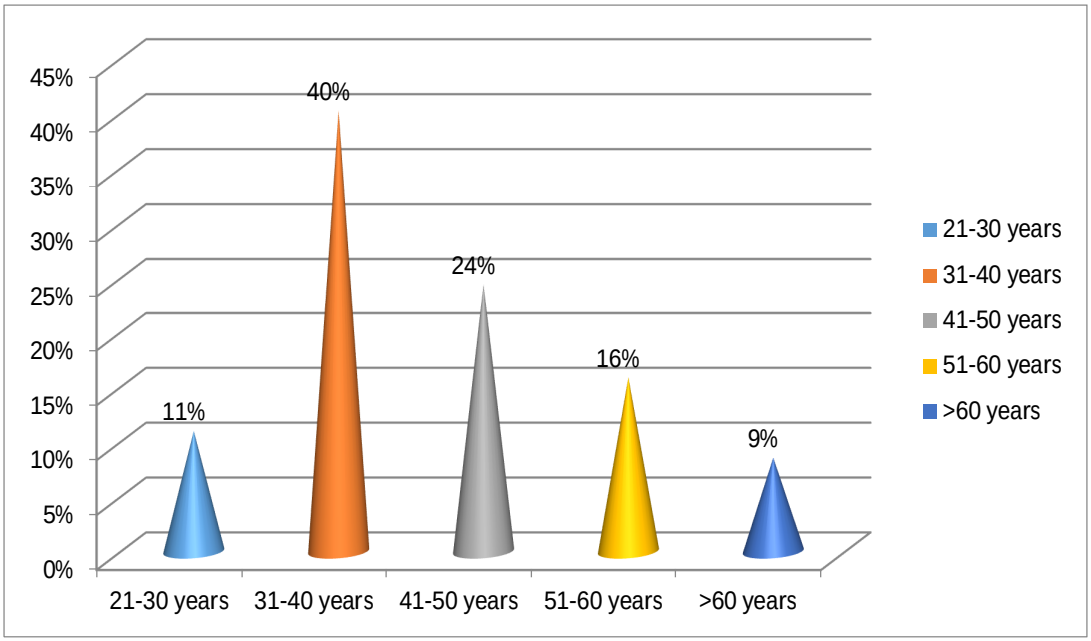


TABLE 12: AGE WISE DISTRIBUTION OF BORDERLINE EPITHELIAL OVARIAN NEOPLASMS

Age group	Number of cases	Percentage
31-40 years	1	25%
41-50 years	2	50%
51-60 years	-	-
>60 years	1	25%
Total cases	4	100%

Maximum incidence of borderline epithelial ovarian neoplasms was found in the age group of 41-50 years. Mean age affected was found to be 47.21 years (Table 12,Chart 9).

CHART 9: AGE WISE DISTRIBUTION OF BORDERLINE EPITHELIAL OVARIAN NEOPLASMS.

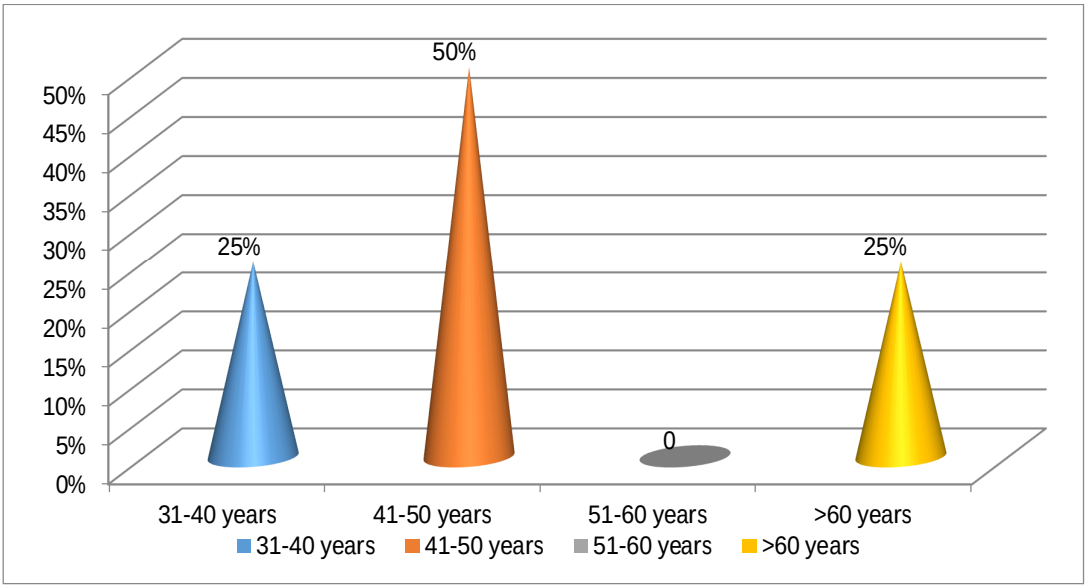


TABLE 13: AGE WISE DISTRIBUTION OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS

Age group	Number of cases	Percentage
41-50 years	8	30.76%
51-60 years	10	38.46%
61-70 years	7	26.92%
>70 years	1	3.8%
Total cases	26	100%

Maximum incidence of malignant epithelial ovarian tumours was found in the age group of 51 to 60 years followed by 41 to 50 years. Mean age affected was found to be 54.5 years. (Chart 10).

CHART 10: AGE WISE DISTRIBUTION OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS

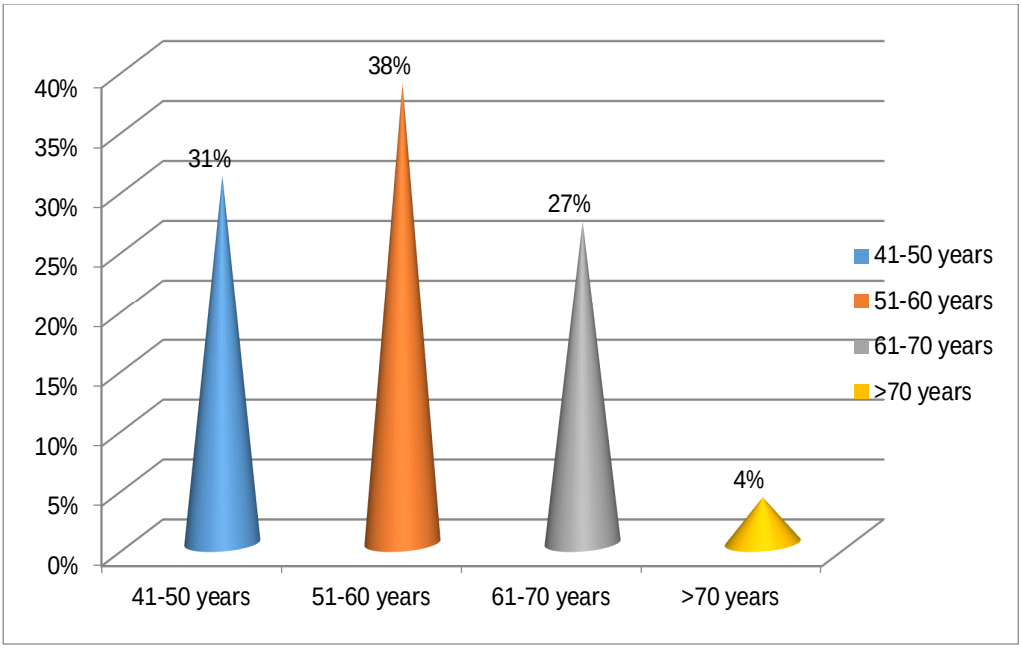


TABLE 14: GRADE WISE DISTRIBUTION OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS

Grade	Number of cases	Percentage
I	5	19.23%
II	9	34.61%
III	12	46.16%
Total cases	26	100%

We can see that maximum tumours were in grade III (Chart 11).

CHART 11: GRADE WISE DISTRIBUTION OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS

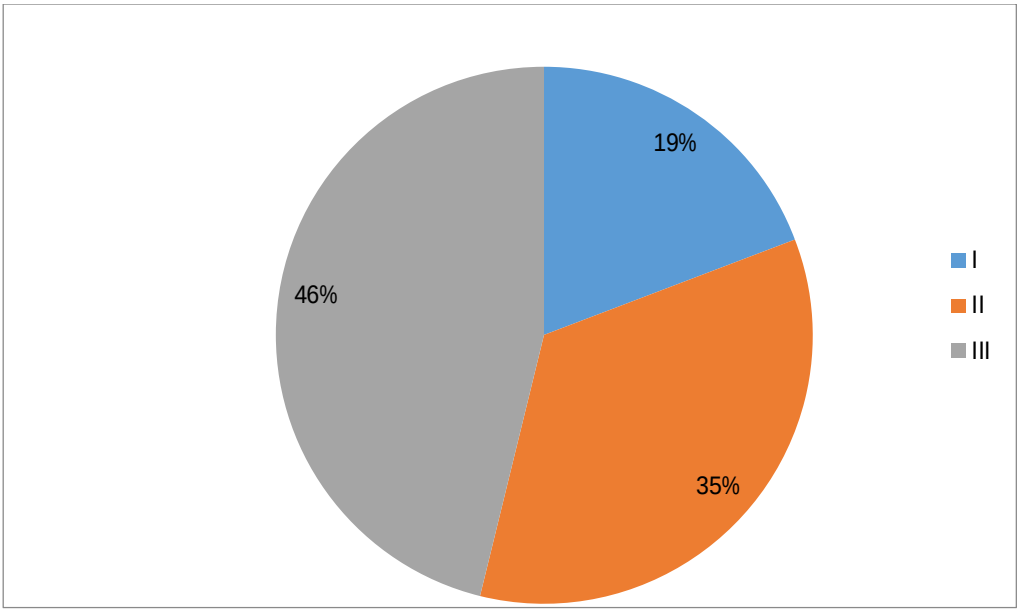


TABLE 15: DISTRIBUTION OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS ACCORDING TO THE FIGO (INTERNATIONAL FEDERATION OF GYNAECOLOGY AND OBSTETRICS) STAGE.

Stage	Number of cases	Percentage
I	4	15.38%
IIA	8	30.76%
IIB	2	7.69%
IIIB	5	19.23%
IIIC	7	26.92%
Total cases	26	100%

CHART 12: DISTRIBUTION OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS ACCORDING TO THE FIGO (INTERNATIONAL FEDERATION OF GYNAECOLOGY AND OBSTETRICS) STAGE.

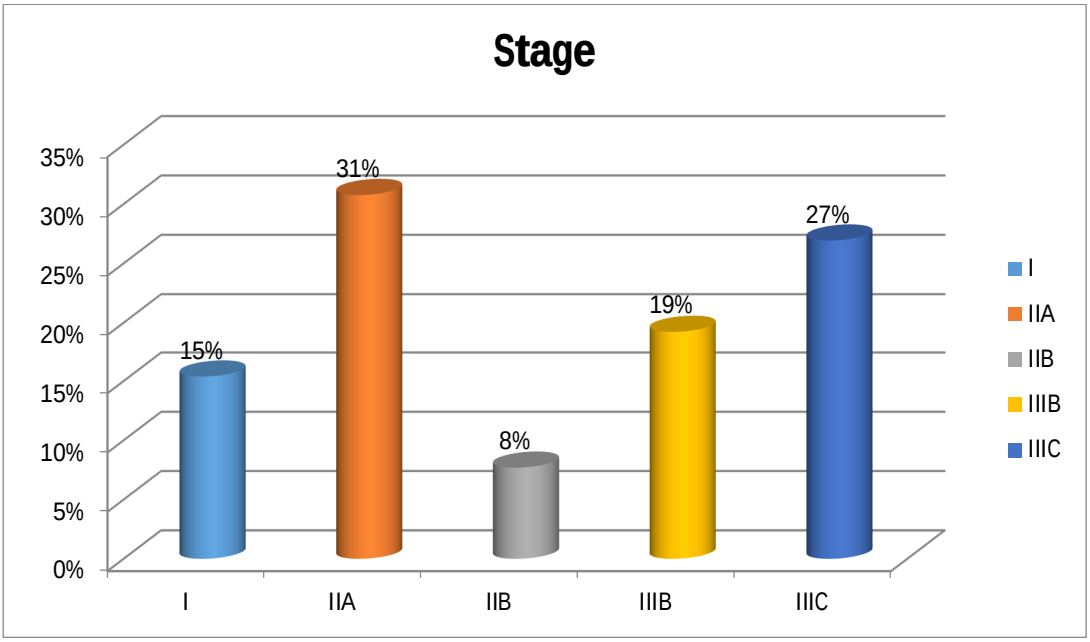
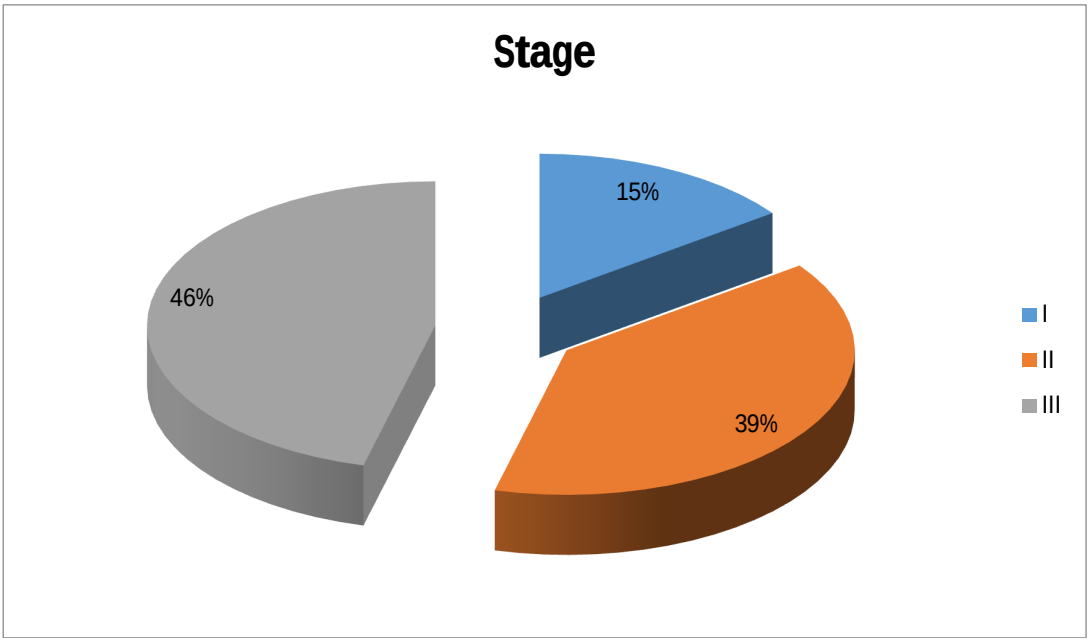


CHART 13: STAGE DISTRIBUTION AMONG MALIGNANT EPITHELIAL OVARIAN NEOPLASMS



Hence, maximum presentation was in stage III (Chart 13).

Results of ImmunohistoChemical Analysis

All the 26 malignant epithelial ovarian neoplasms and 4 borderline epithelial tumors were subjected to a panel of 2 immunohistochemical markers- EGFR(Epidermal Growth Factor Receptor) and VEGF(Vascular Endothelial Growth Factor)

TABLE 16: PERCENTAGE OF POSITIVE EXPRESSION OF EGFR, VEGF AMONG BORDERLINE OVARIAN NEOPLASMS

IHC marker	Positive Cases	Negative Cases
EGFR	2 (50%)	2 (50%)
VEGF	3 (75%)	1 (25%)

Out of the four borderline ovarian neoplasms, 50% showed positivity for EGFR and another 50% showed negativity for EGFR.

Out of four borderline epithelial ovarian neoplasms, 75% showed positivity for VEGF.

TABLE 17: DISTRIBUTION OF POSITIVITY OF EGFR AND VEGF AMONG TYPES OF BORDERLINE EPITHELIAL OVARIAN NEOPLASMS.

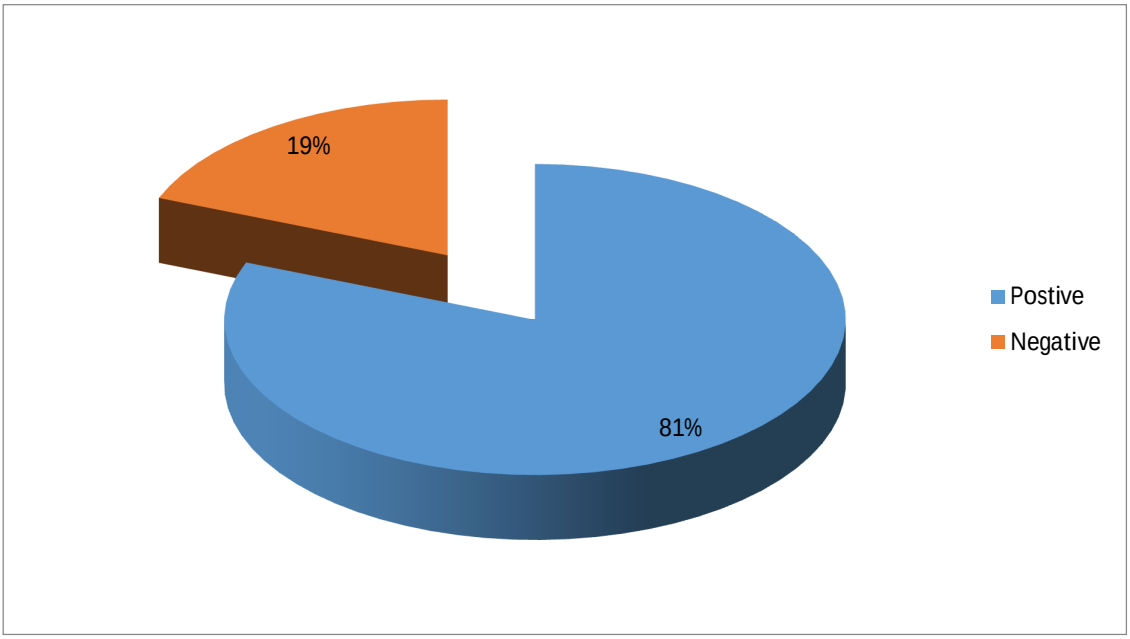
IHC marker	APST Positive (%)	APMT Positive (%)	Total
EGFR	2 (100%)	Nil positive	4
VEGF	2 (100%)	1 (50%)	4

TABLE 18: DISTRIBUTION OF POSITIVITY AMONG MALIGNANT EPITHELIAL OVARIAN NEOPLASMS

IHC marker	Positive cases (%)	Negative cases (%)	Total
EGFR	21 (80.76%)	5 (19.23%)	26 (100%)
VEGF	22 (84.62%)	4 (15.38%)	26 (100%)

Out of the total 26 malignant epithelial ovarian neoplasms, 21 (80.76%) of them showed positivity for EGFR and 19.23% of them were negative for EGFR (Table 18, Chart 14).

CHART 14: DISTRIBUTION OF POSITIVITY AMONG MALIGNANT EPITHELIAL OVARIAN NEOPLASMS



Out of the total 26 malignant epithelial ovarian neoplasms, 22 (84.62%) of them showed positivity for VEGF while only 4 (15.38%) of them were negative for VEGF.

TABLE 19: DISTRIBUTION OF POSITIVITY OF EGFR AND VEGF AMONG TYPES OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS.

Histopathological type of malignant ovarian neoplasm	EGFR Positive	EGFR Negative	VEGF Positive	VEGF Negative	Total
Papillary serous cystadeno carcinoma	8 (88.89%)	1 (11.11%)	8 (88.89%)	1 (11.11%)	9 (100%)
Endometroid adenocarcinoma	7 (87.5%)	1 (12.5%)	7 (87.5%)	1 (12.5%)	8 (100%)
Mucinous adenocarcinoma	2 (50%)	2 (50%)	3 (75%)	1 (25%)	4 (100%)
Clear cell carcinoma	4 (100%)	Nil	4 (100%)	Nil	4 (100%)
Adenosquamous carcinoma	Nil positive		Nil positive		1 (100%)

Thus, we can infer that –

- 88.89% of papillary serous cystadenocarcinoma ovary showed positivity for both EGFR and VEGF.
- 87.5% of endometroid adenocarcinoma ovary showed positivity for both EGFR and VEGF.
- Only 50% of mucinous adenocarcinoma showed positivity for EGFR while 75% of them showed positivity for VEGF
- All the clear cell carcinomas – (100% of them) showed positivity for both EGFR and VEGF
- The adenosquamous carcinoma that was evaluated did not show positivity for either EGFR or VEGF.

TABLE 20: TABLE FOR COMPARISON OF INTENSITY OF EXPRESSION OF EGFR AND VEGF AMONG BORDERLINE TUMORS AND MALIGNANT EPITHELIAL OVARIAN TUMORS.

		EGFR			Total
		NEGATIVE	2+	3+	
MALIGNANT TUMOURS	Count	5	5	16	26
	% within EGFR	71.4%	71.4%	100.0%	86.7%
BORDERLINE TUMOURS	Count	2	2	0	4
	% within EGFR	28.6%	28.6%	0.0%	13.3%
Total	Count	7	7	16	30
	% within EGFR	100.0%	100.0%	100.0%	100.0%

P=0.042

CHART 15: TABLE FOR COMPARISON OF INTENSITY OF EXPRESSION OF EGFR AND VEGF AMONG BORDERLINE TUMORS AND MALIGNANT EPITHELIAL OVARIAN TUMORS.

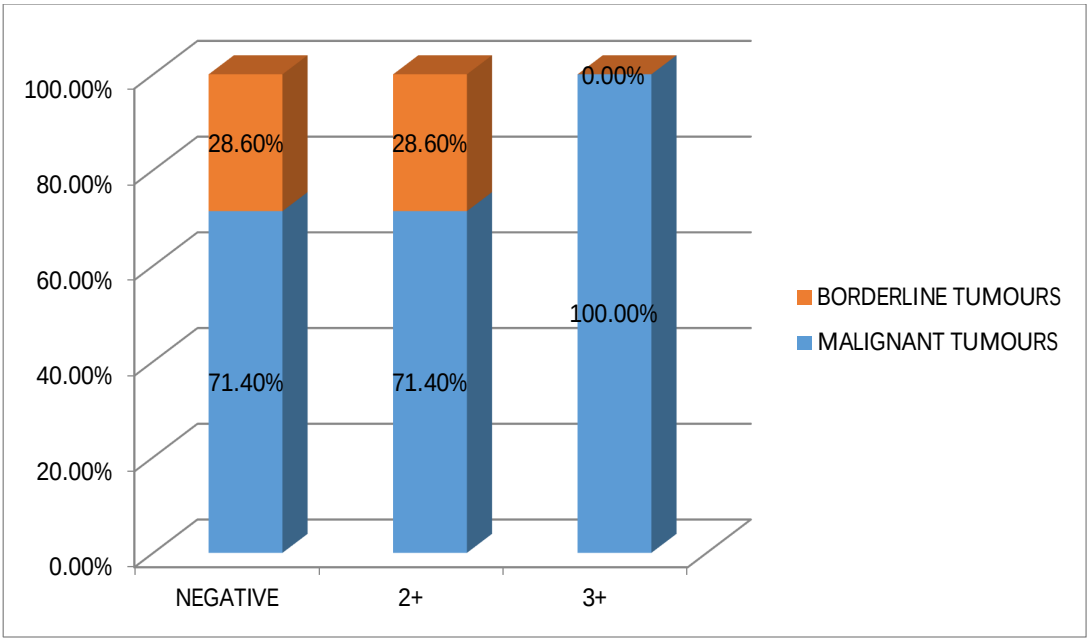
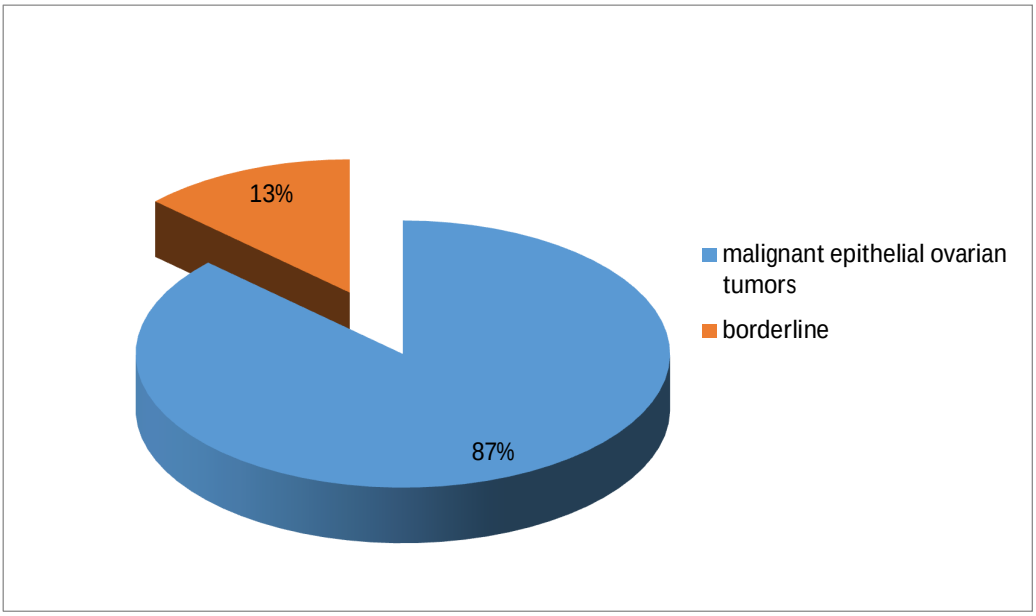


CHART 16: PERCENTAGE OF EGFR POSITIVITY AMONG BORDERLINE AND MALIGNANT EPITHELIAL OVARIAN NEOPLASMS.



From this, we infer that 86.7% of malignant epithelial ovarian tumours showed varying degrees of positivity for EGFR while only 13.3% of borderline epithelial tumours showed positivity. The P value was calculated as 0.042 and hence this correlation was found statistically significant (Table 20,Chart 16.).

TABLE 21: PERCENTAGE OF EXPRESSION OF EGFR IN MALIGNANT EPITHELIAL OVARIAN NEOPLASMS.

HPE		EGFR			Total
		NEGATIVE	2+	3+	
Papillary Serous Cystadenocarcinoma	Count	1	3	5	9
	%	11.11%	33.33%	55.56%	100.00%
Endometrioid adenocarcinoma of ovary	Count	1	2	5	8
	%	12.50%	25.00%	62.50%	100.00%
Mucinous adenocarcinoma ovary	Count	2	0	2	4
	%	50.00%	0.00%	50.00%	100.00%
Clear cell carcinoma ovary	Count	0	0	4	4
	%	0.00%	0.00%	100.00%	100.00%
Adenosquamous carcinoma ovary	Count	1	0	0	1
	%	100.00%	0.00%	0.00%	0.00%
Borderline tumors	Count	2	2	0	4
	%	50.00%	50.00%	0.00%	13.30%
Total	Count	7	7	16	30
	%	23.33%	23.33%	53.33%	100.00%

From this table we infer that nearly 100% of clear cell carcinomas studied, 62.5% of endometroid carcinomas studied, 55.56% of papillary serous carcinomas studied and 50% of mucinous carcinomas studied showed EGFR positivity. (Table 21,Chart 17).

CHART 17: PERCENTAGE OF EXPRESSION OF EGFR IN BORDERLINE TUMORS AND MALIGNANT EPITHELIAL OVARIAN NEOPLASMS.

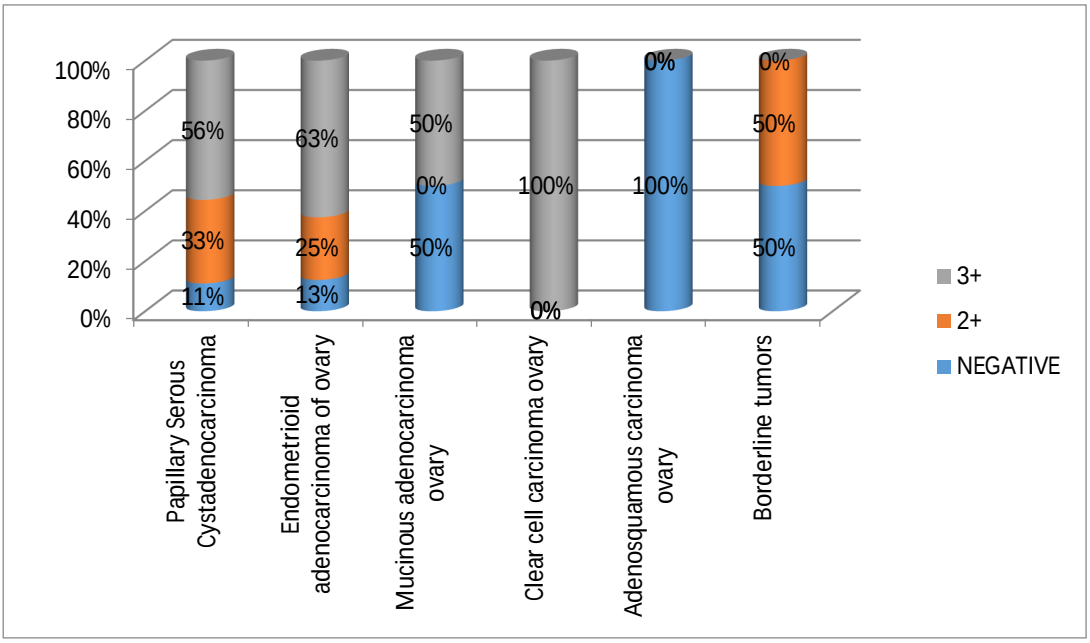


TABLE 22 : CORRELATION OF TUMOR GRADE WITH EGFR EXPRESSION

			EGFR			Total
			NEGATIVE	2+	3+	
Tumor grade	1.00	Count	4	2	0	6
		% within EGFR	57.1%	28.6%	0.0%	20.0%
	2.00	Count	2	5	3	10
		% within EGFR	28.6%	71.4%	18.8%	33.3%
	3.00	Count	1	0	13	14
		% within EGFR	14.3%	0.0%	81.2%	46.7%
Total		Count	7	7	16	30
		% within EGFR	100.0%	100.0%	100.0%	100.0%

P<0.001

In this study, 81.2% of grade III tumours showed 3+ EGFR positivity. Higher the grade, higher was the expression of EGFR and this correlation was statistically highly significant since the P value was less than 0.001(Table 22,Chart 18).

CHART 18: CORRELATION OF TUMOR GRADE WITH EGFR EXPRESSION

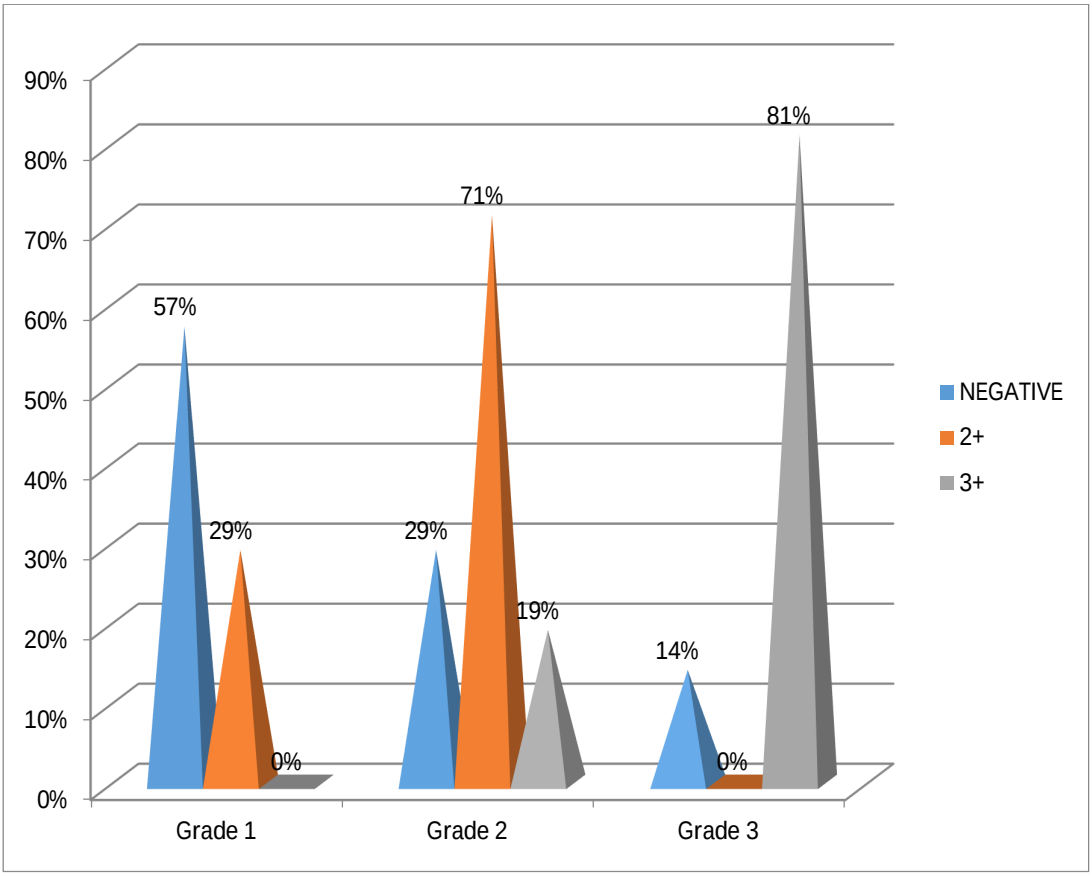


TABLE 23: CORRELATION OF TUMOR STAGE WITH EGFR EXPRESSION

			EGFR			Total
			NEGATIVE	2+	3+	
Stage		Count	2	2	0	4
		% within EGFR	28.6%	28.6%	0.0%	13.3%
	II A	Count	3	3	4	10
		% within EGFR	42.9%	42.9%	25.0%	33.3%
	II B	Count	1	1	0	2
		% within EGFR	14.3%	14.3%	0.0%	6.7%
	III B	Count	0	1	5	6
		% within EGFR	0.0%	14.3%	31.2%	20.0%
	III C	Count	1	0	7	8
		% within EGFR	14.3%	0.0%	43.8%	26.7%
Total		Count	7	7	16	30
		% within EGFR	100.0%	100.0%	100.0%	100.0%

P= 0.039

In this study 75% of stage III tumors showed 3+ positivity. Higher the stage, higher was the expression of EGFR and this correlation was statistically significant since P value was 0.039(Table 23,Chart 19).

CHART 19 CORRELATION OF TUMOR STAGE WITH EGFR EXPRESSION

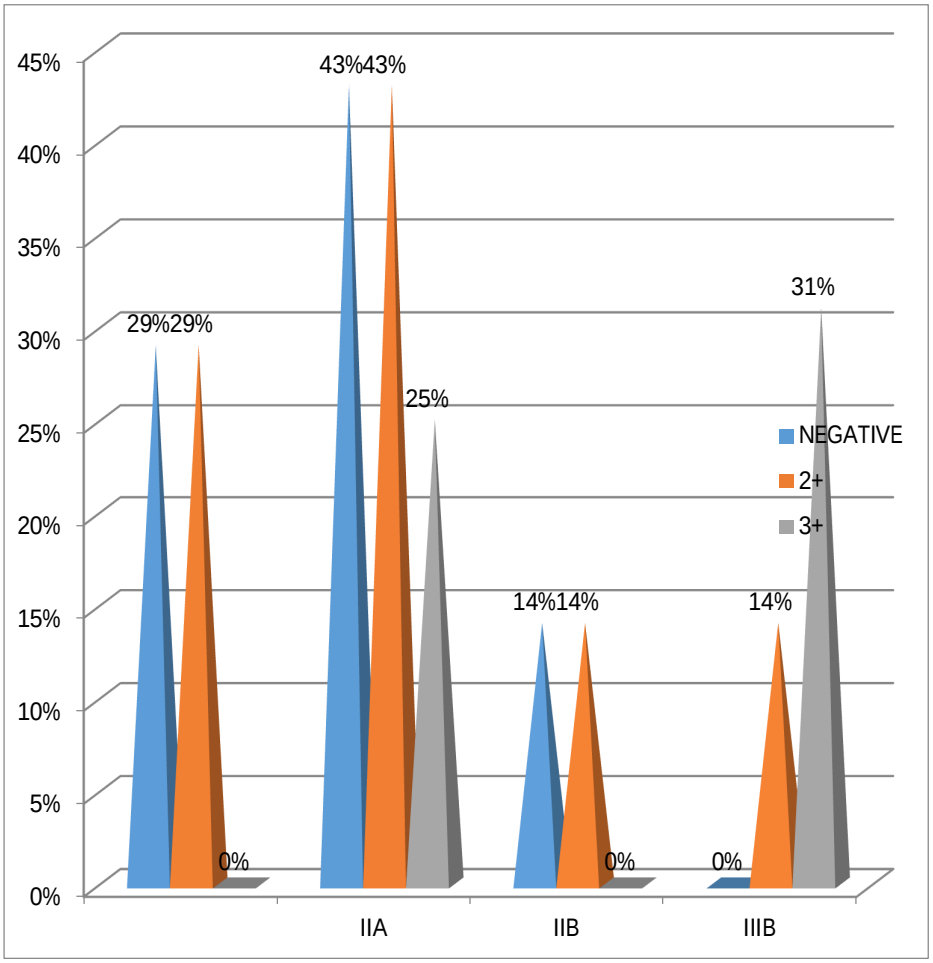


TABLE 24: TABLE FOR COMPARISON OF INTENSITY OF VEGF EXPRESSION AMONG BORDERLINE AND MALIGNANT EPITHELIAL OVARIAN TUMORS

		VEGF				Total
		NEGATIVE	1+	2+	3+	
MALIGNANT TUMOURS	Count	4	0	4	18	26
	% within VEGF	80.0%	0.0%	66.7%	100.0%	86.7%
BORDERLINE TUMOURS	Count	1	1	2	0	4
	% within VEGF	20.0%	100.0%	33.3%	0.0%	13.3%
Total	Count	5	1	6	18	30
	% within VEGF	100.0%	100.0%	100.0%	100.0%	100.0%

P=0.009

From this we infer that 86.7% of malignant epithelial ovarian tumors showed varying degrees of positivity for VEGF while only 13.3% of borderline tumors showed VEGF positivity. The P value 0.009 shows that this correlation was statistically significant (Chart 20).

CHART 20: TABLE FOR COMPARISON OF INTENSITY OF VEGF EXPRESSION AMONG BORDERLINE AND MALIGNANT EPITHELIAL OVARIAN TUMORS

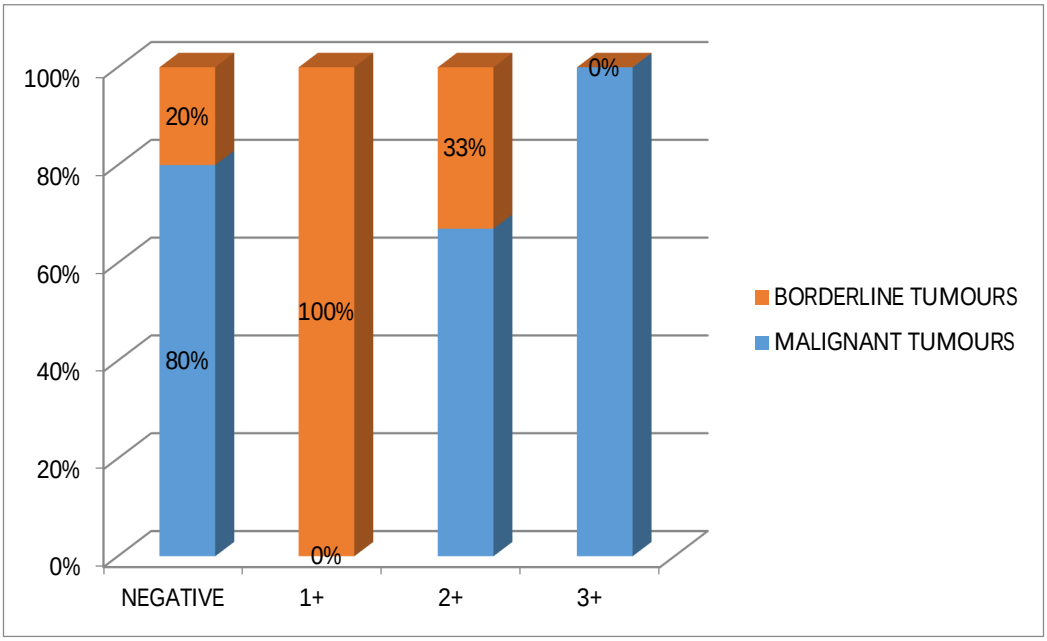


TABLE 25: CORRELATION OF VEGF WITH HISTOPATHOLOGICAL TYPES OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS

			VEGF				Total
			NEGATIVE	1+	2+	3+	
HPE	Papillary Serous Cystadeno carcinoma	Count	1	0	1	7	9
		% within VEGF	11.11%	0.00%	11.11%	77.78%	100.00%
	Endometrioid adenocarcinoma of ovary	Count	1	0	2	5	8
		% within VEGF	12.50%	0.00%	25.00%	62.50%	100.00%
	Mucinous adenocarcinoma ovary	Count	1	0	1	2	4
		% within VEGF	25.00%	0.00%	25.00%	50.00%	100.00%
	Clear cell carcinoma ovary	Count	0	0	0	4	4
		% within VEGF	0.00%	0.00%	0.00%	100.00%	100.00%
	Adenosquamous carcinoma ovary	Count	1	0	0	0	1
		% within VEGF	100.00%	0.00%	0.00%	0.00%	100.00%
	Borderline tumors	Count	1	1	2	0	4
		% within VEGF	25.00%	25.00%	50.00%	0.00%	100.00%
Total		Count	5	1	6	18	30
		% within VEGF	16.67%	3.33%	20.00%	60.00%	100.00%

This table shows that nearly 100% of clear cell carcinomas studied, 77.78% of papillary serous carcinomas studied, 62.5% of endometroid carcinomas studied, and 50% of mucinous carcinomas studied showed VEGF positivity (Chart 19).

CHART 21: CORRELATION OF VEGF WITH HISTOPATHOLOGICAL TYPES OF MALIGNANT EPITHELIAL OVARIAN NEOPLASMS

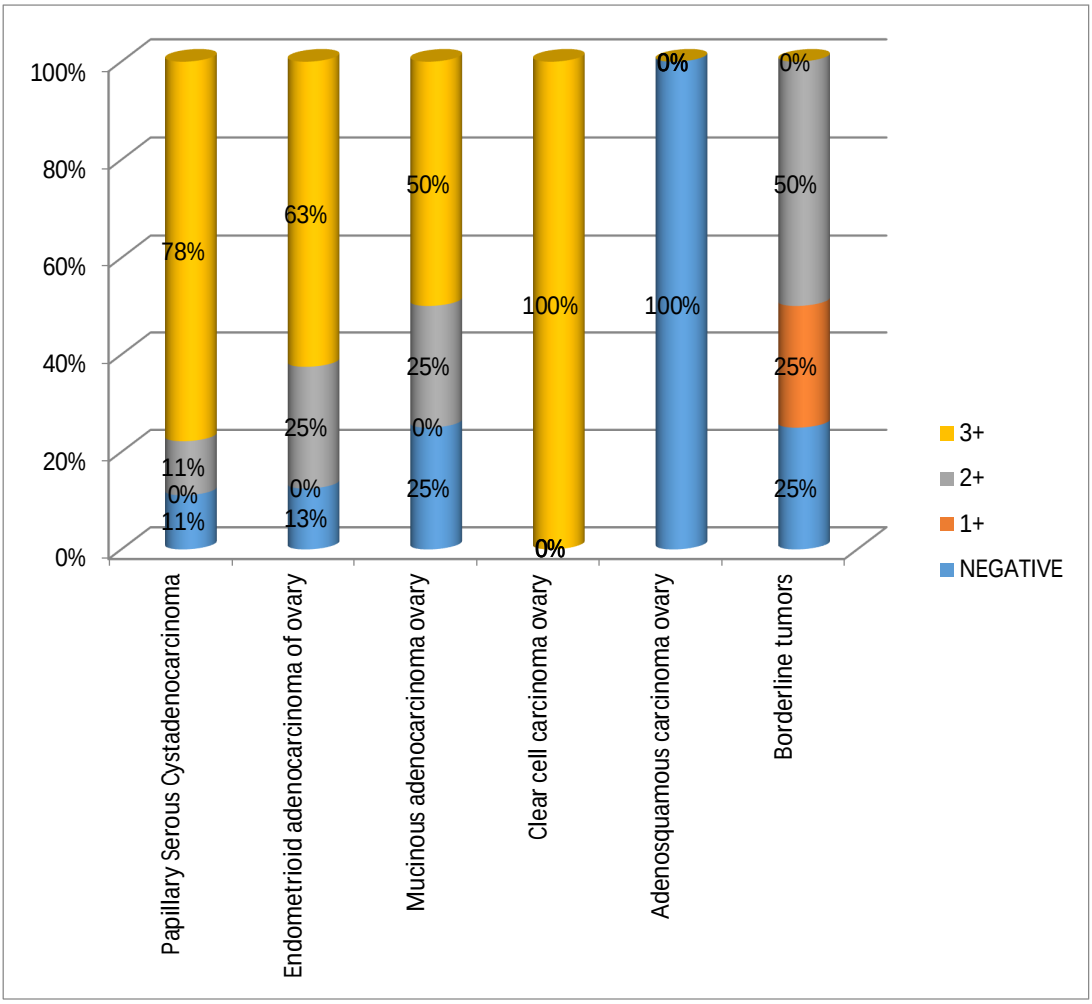


TABLE 26: CORRELATION OF TUMOR GRADE WITH VEGF EXPRESSION

Crosstab							
			VEGF				Total
			NEGATIVE	1+	2+	3+	
Grade	1.00	Count	2	1	3	0	6
		% within VEGF	40.0%	100.0%	50.0%	0.0%	20.0%
	2.00	Count	2	0	3	5	10
		% within VEGF	40.0%	0.0%	50.0%	27.8%	33.3%
	3.00	Count	1	0	0	13	14
		% within VEGF	20.0%	0.0%	0.0%	72.2%	46.7%
Total		Count	5	1	6	18	30
		% within VEGF	100.0%	100.0%	100.0%	100.0%	100.0%

P=0.006.

In this study, 72.2% of grade 3 tumors showed 3+ VEGF positivity. Higher the grade, higher was the expression of VEGF and this correlation was found to be statistically significant as P value was 0.006(Chart 20).

CHART 22 CORRELATION OF TUMOR GRADE WITH VEGF EXPRESSION

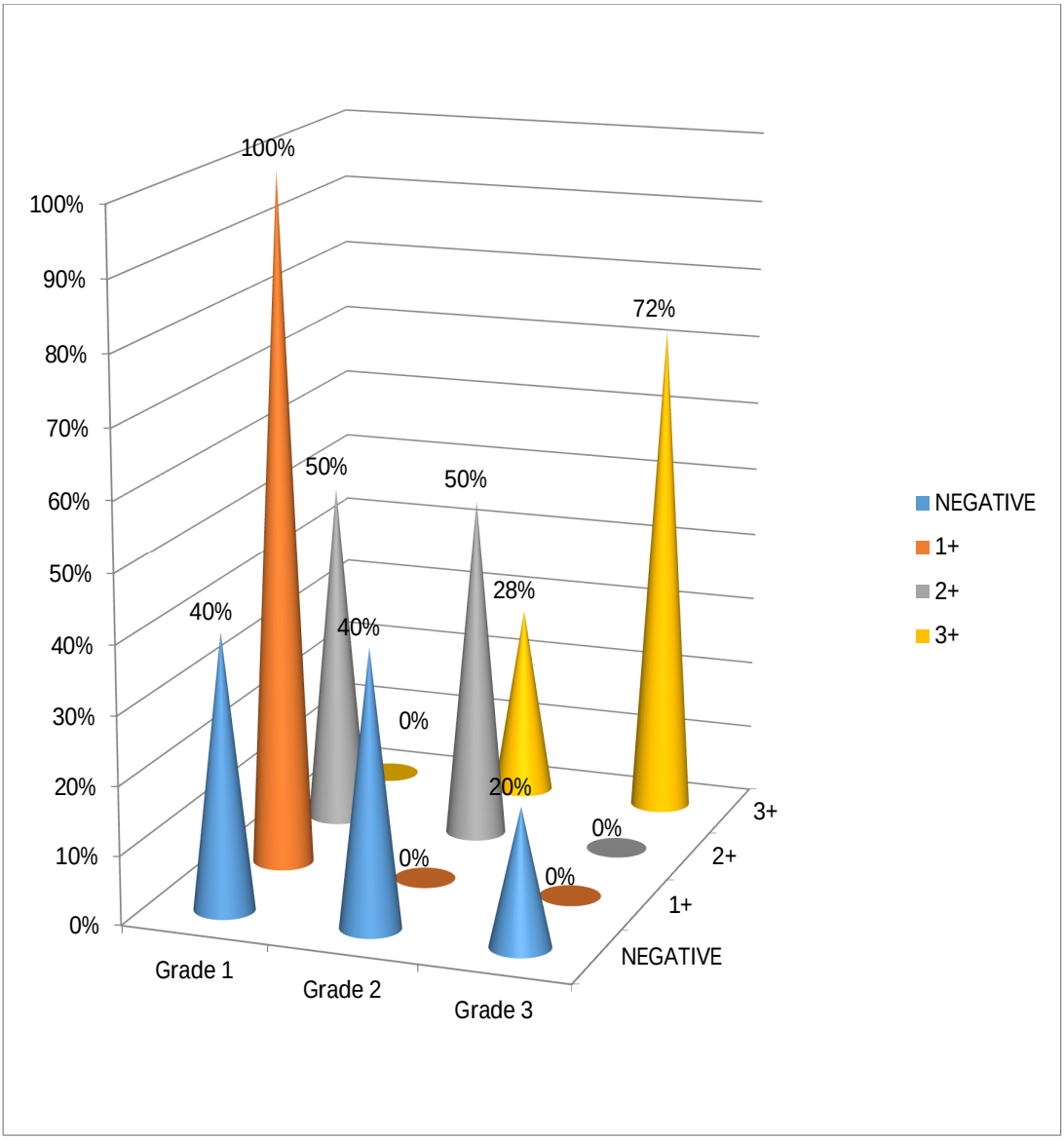


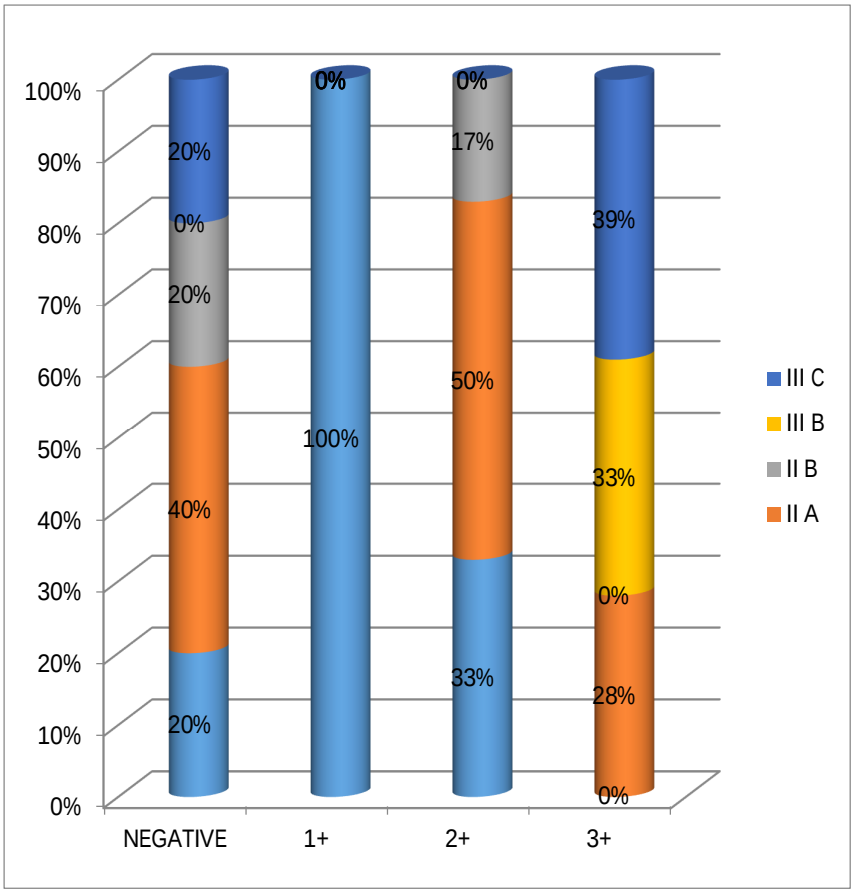
TABLE 27: CORRELATION OF TUMOR STAGE WITH VEGF EXPRESSION

			VEGF				Total
			NEGATIVE	1+	2+	3+	
Stage		Count	1	1	2	0	4
		% within VEGF	20.0%	100.0%	33.3%	0.0%	13.3%
	IIA	Count	2	0	3	5	10
		% within VEGF	40.0%	0.0%	50.0%	27.8%	33.3%
	IIB	Count	1	0	1	0	2
		% within VEGF	20.0%	0.0%	16.7%	0.0%	6.7%
	IIIB	Count	0	0	0	6	6
		% within VEGF	0.0%	0.0%	0.0%	33.3%	20.0%
	IIIC	Count	1	0	0	7	8
		% within VEGF	20.0%	0.0%	0.0%	38.9%	26.7%
Total		Count	5	1	6	18	30
		% within VEGF	100.0%	100.0%	100.0%	100.0%	100.0%

P=0.043

In this study 72.2% of stage III tumors showed 3+ VEGF positivity. Higher the stage, higher was the expression of VEGF and this correlation was found to be statistically significant since P value was 0.043. (Chart 23)

CHART 23: CORRELATION OF TUMOR STAGE WITH VEGF EXPRESSION.



Colour Plates

Figure 1:HPE NO:1842/15,Papillary serous cystadeno carcinoma ovary

Figure 2: HPE NO:3160/15,Endometrioid adenocarcinoma ovary

Figure 3:HPE NO:877/15 Mucinous adenocarcinoma ovary

Figure 4:HPE NO:254/15 Clear cell adenocarcinoma ovary

Figure 5:HPE NO:404/14 Adenosquamous carcinoma ovary.

Figure 6:HPE NO:305/13-Atypical Proliferating Serous Tumour

IHC Profile of papillary serous cystadenocarcinoma

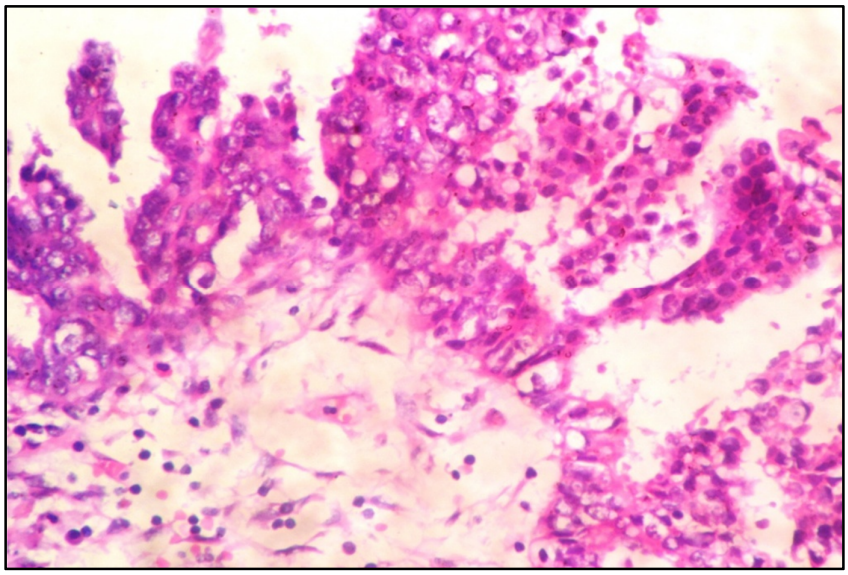


Figure 7: H&E-High power view

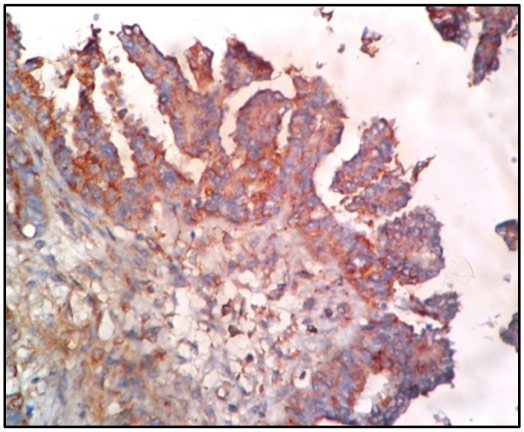


Figure 8:EGFR Score 3+

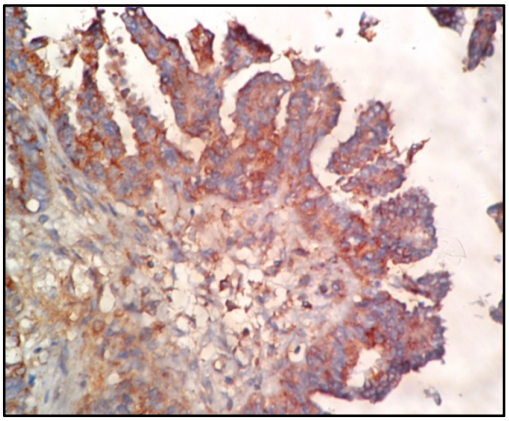


Figure 9:VEGF Score 3+

Papillary serous cystadenocarcinoma

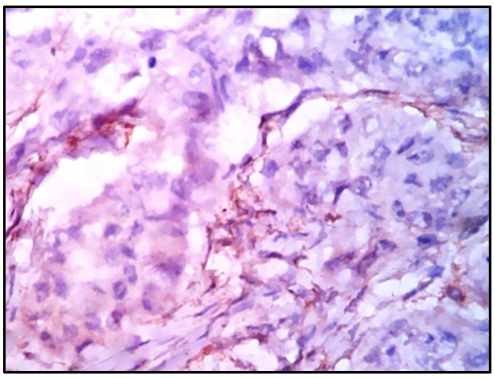


Figure 10:EGFR score 2+

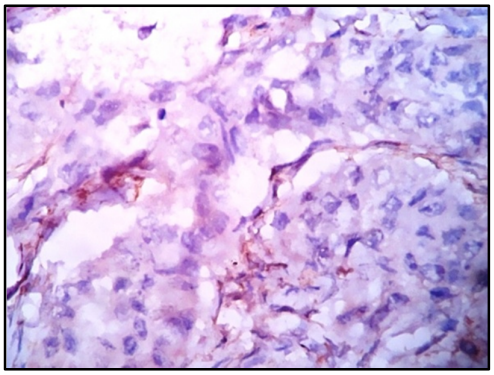


Figure 11:VEGF score 2+

Endometrioid adeno carcinoma

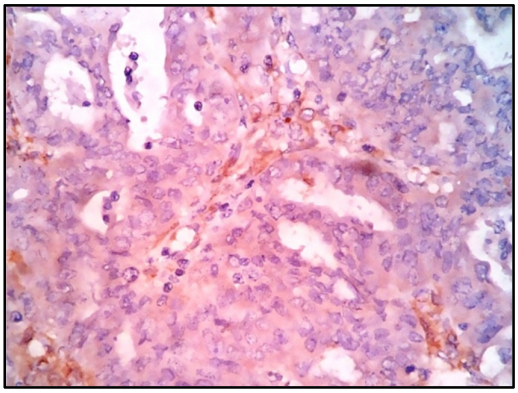


Figure 12:EGFR score 2+

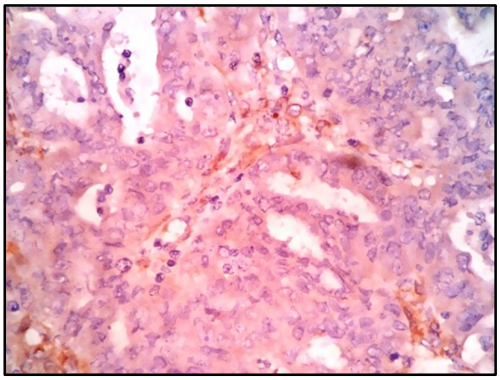


Figure 13:VEGF score 2+

IHC Profile of endometrioid adenocarcinoma ovary

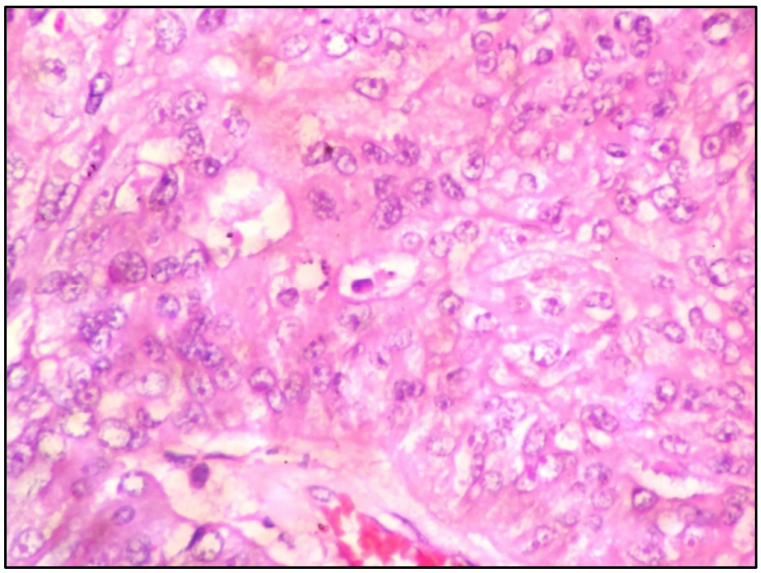


Figure 14:H&E High power view

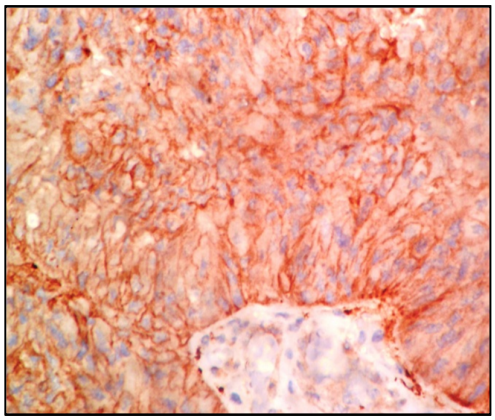


Figure 15:EGFR Score 3+

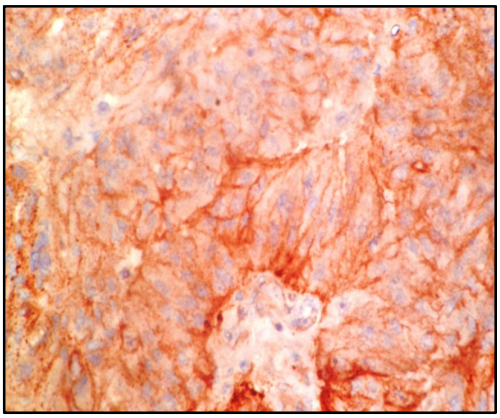


Figure 16:VEGF Score 3+

IHC Profile of mucinous adenocarcinoma ovary

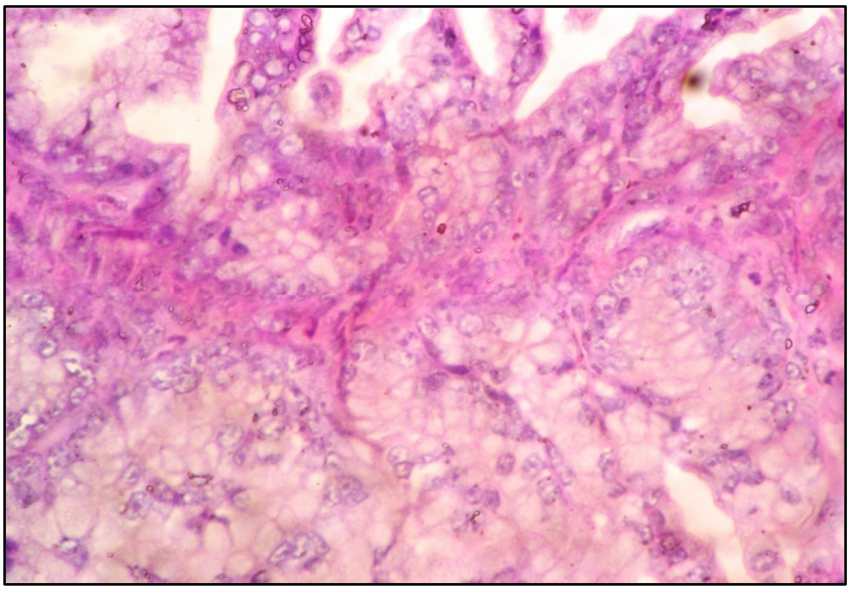


Figure 17:H&E High power view

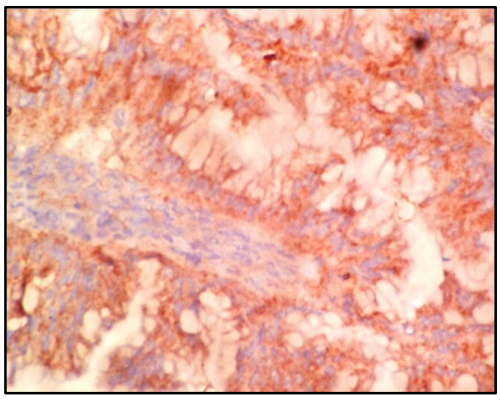


Figure 18:EGFR Score 3+

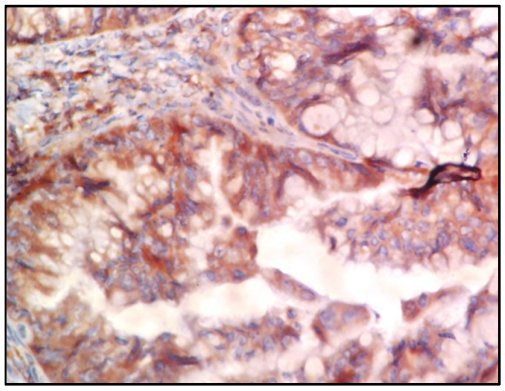


Figure 19:VEGF Score 3+

IHC Profile of clear cell adenocarcinoma

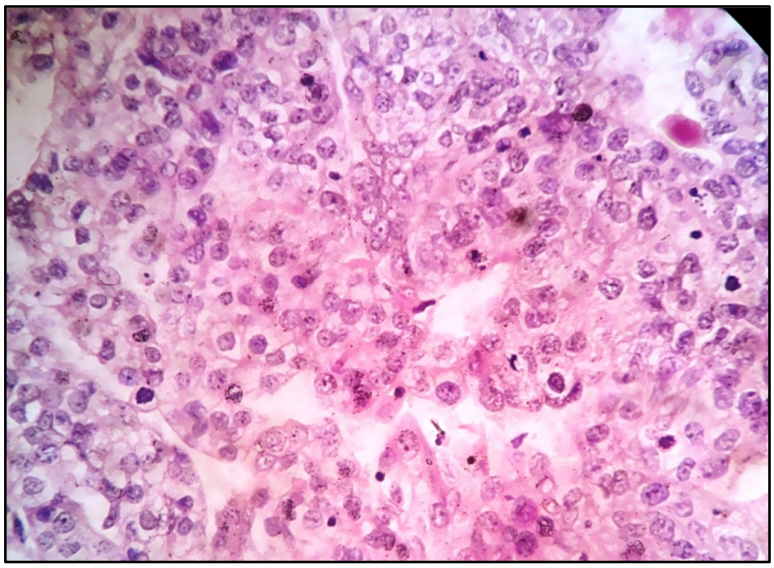


Figure 20:H&E High power view

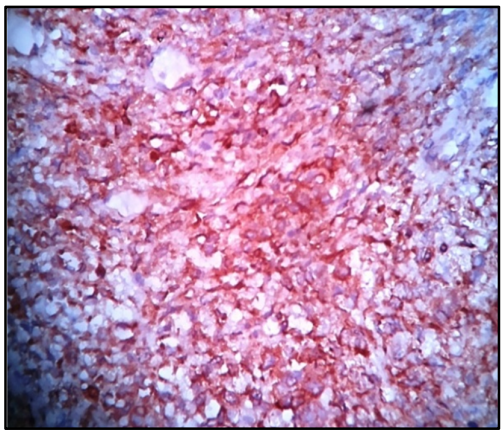


Figure 21:EGFR Score 3+

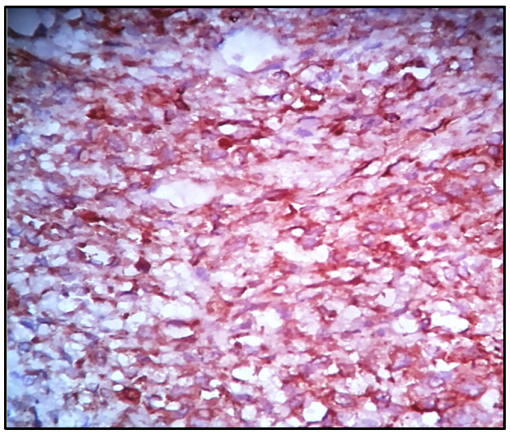


Figure 22:VEGF Score 3+

IHC Profile of adenosquamous carcinoma

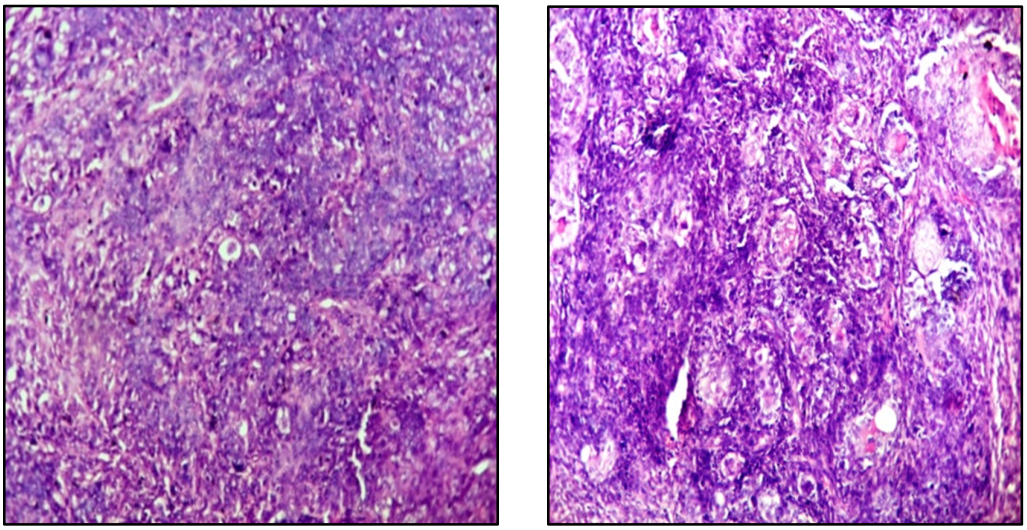


Figure 23:H&E High power view

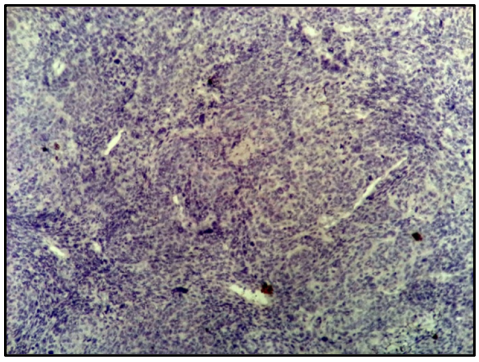


Figure 24:EGFR Negative

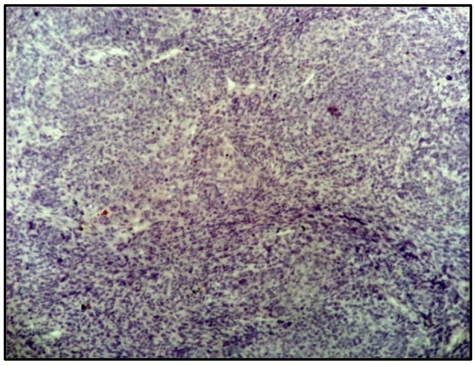


Figure 25:VEGF Negative

IHC Profile of borderline tumors ovary

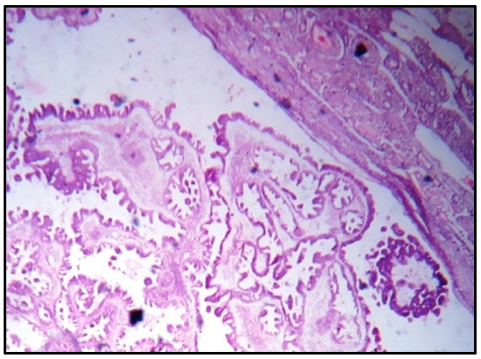


Figure 26:H&E Low power view

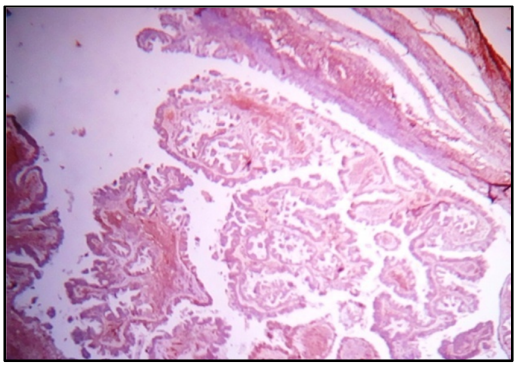


Figure 27:EGFR Positive, low power view

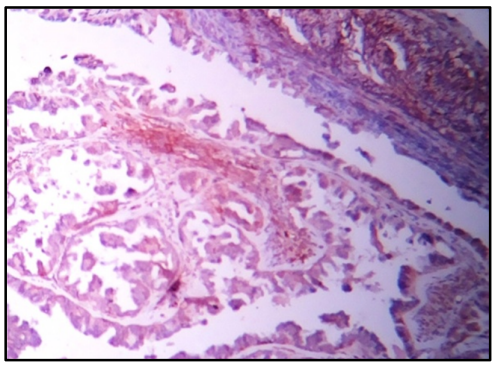


Figure 28:VEGF Positive, low power view

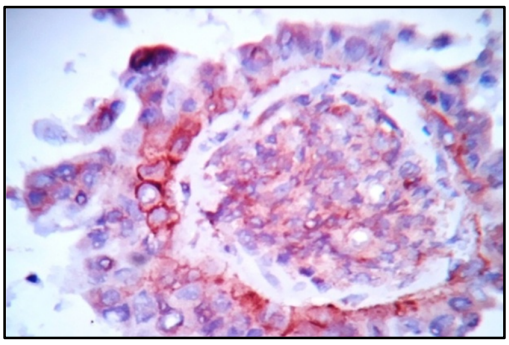


Figure 29:EGFR Score 3+ High power view

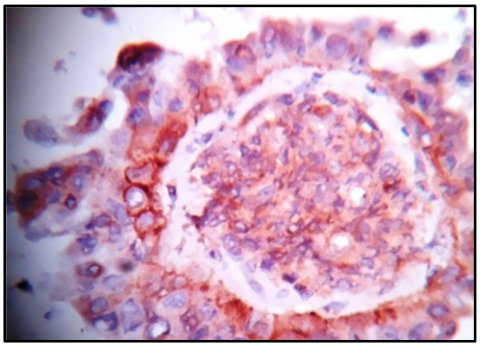


Figure 30:VEGF Score 3+ High power view

Discussion

DISCUSSION

Carcinoma ovary is the fourth leading cause of death among women. Amidst neoplasms of female genital tract, ovarian carcinoma carries maximum morbidity and mortality as there are no easy or effective screening techniques and most of the ovarian neoplasms present at advanced stage,since early stages are predominantly asymptomatic.

In the study by Vijaykumar et at [109], involving 150 cases of ovarian specimen sent for histopathology at Govt Medical College, Patiala Punjab, 40% (60 cases) were neoplastic and 60% (90 cases) were non neoplastic

In the study by Layla et al [110], on pattern of ovarian neoplasms in Saudi Arabia, out of 618 ovarian specimen studied, 61.8% were ovarian neoplasms while 38.2% were non neoplastic

In a study of 145 cases by Kanithkar et al from Maharashtra, 75% were non neoplastic and 25% were neoplastic.

In our current study involving 2435 ovarian specimen, 34.74% were non-neoplastic, while 7.02% were neoplastic.

TABLE 28 – PERCENTAGE OF NEOPLASTIC AND NON NEOPLASTIC OVARIAN LESIONS IN VARIOUS STUDIES

Study	Non neoplastic percentage	Neoplastic percentage
Vijaykumar et al (150 cases)	60%	40%
Layla et al (618 cases)	38.2%	61.8%
Kanithkar et al (145 cases)	75%	25%
Current study	34.74%	7.02%

In the sudy by Prakashiny et al [112] involving 80 cases of ovarian neoplasms, benign ovarian tumors accounted for 72.5% while malignant tumors accounted for 27.5%.

In the study by GG Swamy et al involving 120 cases, 86 were benign and 24 were malignant, constituting 71.6% and 28.4% respectively.

In the study by Kanithkar et al involving 145 cases, 78.57% were benign while 21.43% were malignant [111]

In the study by Mankar DV et al of the total 257 cases of ovarian tumors, 63.04% were benign while 36.96% were malignant [114]

In the study by Layla et al [110], of the 382 ovarian neoplasms, benign ones constituted 72.8% while malignant ones were about 27.2%.

While in our current study of 171 ovarian neoplasms 71.1% were benign, while 28.9% were malignant.

TABLE 29 – PERCENTAGE OF BENIGN AND OVARIAN NEOPLASMS IN VARIOUS STUDIES

Study	Benign ovarian neoplasms' percentage	Malignant ovarian neoplasms' percentage
Prakashiny et al	72.5%	27.5%
GG Swamy et al	71.6%	28.4%
Kanithkar S.N et al	78.57%	28.43%
Mankar DV et al	63.04%	36.90%
Layla et al	72.8%	27.2%
Current study	71.1%	28.9%

In the study by Prakashiny et al [112], of 80 cases of ovarian neoplasms, surface epithelial ovarian tumors constituted 61.25% of the total.

The study by GG Swamy et al [113] of 120 cases of ovarian neoplasms showed that 61.6% of them were of surface epithelial type.

Kanithkar SN [111] et al studied 145 cases and showed that, surface epithelial ovarian tumors were commonest (67.14%) [111]

In the study by Mankar DV et al [114], 257 cases of ovarian tumors were studied of which 68.48% were surface epithelial ovarian neoplasms.

In our current study, of the 171 ovarian neoplasms, 53.801% were of surface epithelial type.

TABLE 30 – COMPARISON OF PERCENTAGE OF SURFACE EPITHELIAL OVARIAN NEOPLASMS AMONG TOTAL IN VARIOUS STUDIES:

Study	Surface epithelial ovarian neoplasms' percentage
Prakashiny et al	61.25%
GG Swamy et al	61.6%
Kanithkar S.N et al	67.14%
Mankar DV et al	68.48%
Layla et al	61%
Current study	53.801%

In the study by Prakashiny et al [112], simple serous cystadenoma (52.5%) accounted for majority of benign epithelial tumors among a total of 80 ovarian lesions.

In the study by GG Swamy et al [113] of 120 cases, the most common benign epithelial tumor was noted to be serous cystadenoma (40.8%).

Kanithkar SN [111] et al in his study of 145 ovarian neoplasms, found that the majority of benign epithelial tumors were of benign serous cystadenoma category constituting about 39.8%.

In the study of 257 cases of ovarian tumors by Mankar DV et al [114], mucinous cystadenoma topped the list of benign epithelial tumors.

Layla et al [110] studied 382 cases of ovarian tumors and concluded that serous cystadenoma formed the major bulk of benign epithelial tumors constituting (34.48%) of total benign epithelial tumors.

In our current study, serous cystadenoma was found to be the most frequent benign epithelial tumor constituting 61.28% of total benign epithelial tumors.

TABLE 31 – THE MOST COMMON BENIGN EPITHELIAL TUMOR IN VARIOUS STUDIES

Study	Most common benign epithelial tumor
Prakashiny et al	Benign serous cystadenoma
GG Swamy et al	Benign serous cystadenoma
Kanithkar S.N et al	Benign serous cystadenoma
Mankar DV et al	Mucinous cystadenoma
Layla et al	Serous cystadenoma
Current study	Serous cystadenoma

In the study by Prakashiny et al [112], serous papillary cystadenocarcinoma (35.6%) formed majority of surface epithelial malignancies out of a total of 80 cases.

The study by GG Swamy et al [113] of 120 cases, the most common malignant epithelial tumor was noted to be endometrioid adenocarcinoma (40.8%).

Kanithkar SN [111] et al in his study of 145 ovarian neoplasms, found that the majority of malignant epithelial ovarian tumors were of serous cystadenocarcinoma category constituting about 37.67% of all malignant epithelial ovarian tumors.

In the study of 257 cases of ovarian tumors by Mankar DV et al [114], serous cystadenocarcinoma (31.13%) topped the list of malignant epithelial ovarian tumors.

Layla et al [110] studied 382 cases of ovarian tumors and concluded that serous cystadenocarcinoma formed the major bulk of malignant epithelial tumors constituting about 38.84% of total malignant epithelial tumors.

In our current study, papillary serous cystadenocarcinoma constituted the major bulk of malignant epithelial tumors.

TABLE 32:MOST FREQUENT MALIGNANT EPITHELIAL TUMOR IN VARIOUS STUDIES.

Study	Most frequent malignant epithelial tumor
Prakashiny et al	Serous papillary cystadenocarcinoma
GG Swamy et al	Endometrioid adenocarcinoma
Kanithkar S.N et al	Serous cystadenocarcinoma
Mankar DV et al	Serous cystadenocarcinoma
Layla et al	Serous cystadenocarcinoma
Current study	Serous cystadenocarcinoma

In this study, Immunohistochemical evaluation was attempted on the 30 borderline and malignant epithelial ovarian neoplasms received at the institute of social obstetrics and Government Kasturba Gandhi Hospital for women and children. Madras Medical College, Chennai in the 3-year period between June 2012 and June 2015.

- Comparison of age distribution of benign epithelial ovarian tumors in the present study and other studies.

Age group

In the study by Verma and Bhatia et al (1981), Inamdar et al (2015) maximum incidence of benign epithelial ovarian tumors was found at age group of 21 to 40 years which is similar to the current study [71](Table 26).

TABLE 33: COMPARISON OF AGE AMONG BENIGN TUMORS

STUDY	AGE GROUP WITH MAXIMUM INCIDENCE
Inamdar et al (2015)(87 cases)	21 – 40 years
Verma et al (1981)(103 cases)	21 – 40 years
Current study	21-40 years

In the study by Jagadeshwari et al (1971) [72] by Bhatia et all (1981) [73] and Inamdar et al (2015) maximum incidence of malignant epithelial ovarian neoplasms was found at age range of 41 to 60 years which is similar to the current study (Table 34).

TABLE 34: COMPARISON OF AGE AMONG MALIGNANT EPITHELIAL OVARIAN TUMORS.

STUDY	AGE GROUP WITH MAXIMUM INCIDENCE.
Jagadeshwari et al (1971)(56 cases)	41 – 60 years
Bhatia et all (1981)(79 cases)	41 – 60 years
Current Study(30 cases)	41 – 60 years

Tumor grade

In the study by Bashir Ahmad et al, poorly differentiated (grade III) tumors topped the list among different, malignant epithelial ovarian neoplasms, constituting 48.4%,which are similar to the current study where the frequency of grade 3 tumors was found to be 46.7%.

TABLE 35 : PERCENTAGE OF GRADE III TUMORS IN VARIOUS STUDIES

STUDY	PERCENTAGE OF GRADE III TUMOURS
Bashir Ahmed et al [74] (64 cases)	48.4%
Current Study (30 cases)	46.7%

Tumor stage

In the study by Mohamed Farouk et al, percentage of epithelial ovarian carcinomas presenting at Stage III was given as 50.6% [75,76] which are similar to the current study where maximum cases presented in the late stage III constituting about 46.15%.

TABLE 36: PERCENTAGE OF MALIGNANT EPITHELIAL OVARIAN TUMORS PRESENTING AT STAGE III IN DIFFERENT STUDIES.

Current Study	Mohamed et al (2012)	Michelle et al (2009)
46.15% (30 cases)	50.6% (53 cases)	61.01% (44 cases)

Immunohistochemical Analysis

Structurally EGFR is made of an extracellular ligand binding domain, a single transmembrane spanning region, and an intracellular region containing the kinase activity [77,78] Activation of EGFR causes transduction of EGFR signals via MAPK (Mitogen Activated Protein Kinase) and AKT (protein Kinase B) pathways, triggering a number of cellular responses like proliferation, differentiation, cell motility and survival [79,80]. The chromosome 7p12 contains the EGFR gene which is expressed in most of ovarian carcinomas and is associated with poor prognosis. [81,82].

The epithelial lining of the ovary normally has weak EGFR expression.Epithelial ovarian neoplasms including borderline and epithelial ovarian carcinomas show EGFR overexpression ranging from(4% to 60%) and (30% to 100% of cases) respectively.[83,84] while in our study, 50% of borderline epithelial ovarian neoplasms and 80.76% of malignant epithelial ovarian neoplasms showed EGFR expression. Similar to breast and bladder cancer, expression of EGFR in ovarian cancer appears to be a poor prognostic factor [85]. EGFR amplification was found to increase with grade of ovarian

tumour [86]. EGFR overexpression has been found to be associated with high tumour grade and stage, high cell proliferation index and poor survival rate for the patient [87]. The percentage of ovarian epithelial cancers overexpressing EGFR range from 63% to 100% (Alper et al 2001, Sewell et al 2002, Skirnisdottir et al 2001) [88]. Even in our current study 80.76% of malignant epithelial ovarian tumours showed EGFR positivity. In our current study, statistically significant correlation existed between EGFR expression and tumour grade and stage as the P value was 0.001 and 0.039 respectively

TABLE 37: PERCENTAGE OF EPITHELIAL OVARIAN CANCERS OVEREXPRESSING EGFR IN VARIOUS STUDIES [88]

STUDY	PERCENTAGE
Alper et al (2001) (64 cases)	72%
Sewell et al (2002) (49 cases)	63%
Skirnis dotit et al (2001) (22 cases)	65%
Current study (2015) (30 cases)	80.76%

VEGF

The dimeric glycoprotein Vascular Endothelial Growth Factor(VEGF) has got potent mitogenic effect on endothelial cells. It plays an important role in regulation of angiogenesis process during embryogenesis. It also plays a vital role in cancer – neoangiogenesis [89]. VEGF is a multifunctional cytokine that causes increase in microvascular permeability and density, nourishing the highly metabolic tumor cells and also provides access to the host vasculature[89].

In the study by Jun Wang et al, only 30% of borderline epithelial ovarian tumours and 80% of malignant epithelial ovarian tumours were positive for VEGF expression [89].

In the study by S.Yamamoto et al, 97% of ovarian carcinomas showed positive immunostaining for VEGF while 52% of borderline epithelial ovarian tumours showed positivity [90]

In the study by Hel, Zhao X et al, 80% of ovarian carcinomas, 21% of borderline epithelial ovarian tumours showed positive VEGF immunostaining [91].

In our present study, positive VEGF expression was found in 75% of borderline and 84.62% of malignant epithelial ovarian tumors.

TABLE 38	**VEGF Positivity**	
Study	**Percentage positivity in**	
	Borderline epithelial tumors	**Malignant ovarian epithelial tumors**
Jun Wang et al(27 cases)	30	80
Yamomoto et al(55 cases)	52	97
Hel, Zhao X et al(37 cases)	21	80
Current Study(30 cases)	75	84.62

VEGF expression showed statistically significant correlation between FIGO stage and lymph node metastasis [91]

VEGF gene expression was positively correlated with Stage III & IV ovarian epithelial cancers [92]

Even in our present study, there was a positive correlation of VEGF with tumor grade or stage with P value of 0.006 and 0.043 respectively.

Summary

SUMMARY

- For the study period of 3 years from 2012 to 2015, total of 9313 histopathological specimens were received at Department of pathology, Institute of social obstetrics and Government Kasturba Gandhi Hospital for women and children.
- Amidst 9313 cases, 2435 were ovarian specimens.
- Amidst 2435 ovarian specimen,1418 were normal,846 were non neoplastic,171 were neoplastic.
- Amidst 171 ovarian neoplasms, 92 (53.81%) were surface epithelial ovarian neoplasms.
- Out of 92 surface epithelial ovarian neoplasms, 62 were benign, 4 were borderline and 26 were epithelial ovarian malignancies.
- Out of the 62 benign ovarian neoplasms, 61.3% were that of benign serous cystadenoma and papillary serous cystadenoma, 30.64% were that of benign mucinous cystadenoma and only 8.06% were of benign Brenner type.
- Out of 4 borderline tumors, 2 were that of atypical proliferating serous tumors and 2 were that of atypical proliferating mucinous tumors.
- Amidst the 26 epithelial ovarian carcinomas, papillary serous cystadenocarcinomas topped the list constituting about 34.61% of total, followed closely by endometrioid adenocarcinomas that constituted 30.76%

of the total followed by clear cell carcinoma and adenosquamous carcinoma.

- Maximum incidence of benign neoplasms was found to be in age group of 31 to 40 years. Peak incidence of borderline ovarian tumors was found in 41 to 50 years age group. 51 to 60 years age group showed maximum incidence of malignant epithelial ovarian neoplasms.
- Amidst total malignancies, grade III tumors contributed maximum percentage with 46.14%
- Maximum tumors presented at an advanced stage - stage III-constituting 46.15% of them.
- Out of 4 borderline ovarian neoplasms, 50% showed positivity for EGFR while 75% of them showed positivity for VEGF.
- Among malignancies, 80.76% of them showed EGFR positivity while 84.02% showed VEGF positivity.
- Amidst malignancies, nearly 100% of clear cell carcinomas, 50% of mucinous carcinomas, and 87.5% of endometrioid adenocarcinomas while 88.89% of papillary serous carcinomas showed positive immunostaining for EGFR.
- Similarly nearly 100% of clear cell carcinomas, 88.89% of papillary serous carcinomas,87.5% of endometrioid adenocarcinomas, 75% of mucinous carcinoma showed positive immunostaining for VEGF.
- The Adenosquamous carcinoma that was evaluated showed neither positivity for EGFR nor VEGF.

- While only 13.3% of borderline epithelial tumors showed EGFR positivity 86.7% of carcinomas showed varying degrees of EGFR positivity. P value 0.042 shows that this correlation was statistically significant.
- 81.2% of grade III tumors showed 3+ EGFR positivity. EGFR expression increased with grade of the tumor and this correlation was found statistically significant since the P value was 0.001.
- 75% of stage III tumors showed 3+ EGFR positivity. EGFR expression was increased with stage of the tumor and this correlation was found statistically significant since the P value was 0.039.
- 85.7% of malignant epithelial neoplasms showed varying degrees of positivity for VEGF while only 14.3% of borderline tumors showed VEGF positivity. P value 0.009 shows that the correlation was statistically significant.
- 72.2% of grade 3 tumors showed 3+ VEGF positivity. Higher the grade, higher was the expression and this correlation was found statistically significant with P value 0.006.
- 72.2% of stage III tumors showed 3+ VEGF positivity. Advanced stage tumors showed increased expression of VEGF and this correlation was statistically significant with P value calculated as 0.043.

Conclusion

CONCLUSION

To conclude, we can say that like all other studies even in this study – the surface epithelial ovarian neoplasms were found to be statistically the most significant one contributing 53.81% of the total ovarian neoplasms. Even among surface epithelial tumours, benign neoplasms significantly outnumbered the borderline and the malignant ones and they mostly occurred in the 30 to 40 years age group. Malignant surface epithelial ovarian tumours showed peak incidence in post-menopausal age group of 50 to 60years. As in the society even in this study, maximum cases presented at an advanced stage III.

With Immunohistochemical analysis, the percentage of EGFR and VEGF expression showed a significant increase in malignant compared to borderline tumours. Even among malignancies, EGFR and VEGF showed a significant correlation with tumour grade and FIGO stage. High grade and advanced stage tumours showed EGFR and VEGF overexpression compared to low grade and early stage carcinomas.

EGFR and VEGF both have diagnostic and therapeutic implications. Both markers were found to be independent prognostic factors in ovarian neoplasms. This is just a hospital based study that may not reflect the exact occurrence in the community as a whole. There is a wide arena of community based studies and research activities being carried out with these two markers - opening up newer dimensions and horizons in the ‘early diagnosis’ and

‘chemotherapeutic approaches’ with anti EGFR and VEGF antibodies in the battle against this ‘silent killer’ called ‘Cancer Ovary’.

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Annexures

ANNEXURE 1

WHO CLASSIFICATION OF OVARIAN TUMORS

TABLE 31.1 WHO Histological Classification of Tumors of the Ovary

Surface epithelial–stromal tumors

Serous tumors

Malignant

Adenocarcinoma

Borderline tumor

Benign

Cystadenoma, adenofibroma, cystadenofibroma

Mucinous tumors

Malignant

Adenocarcinoma

Borderline tumor

Benign

Cystadenoma, adenofibroma, cystadenofibroma

Mucinous cystic tumor with pseudomxoma peritonei

Endometrioid tumors including variants with squamous differentiation

Malignant

Adenocarcinoma

Malignant mixed müllerian tumor (carcinosarcoma)

Endometrioid stromal sarcoma (low grade)

Undifferentiated ovarian sarcoma

Borderline tumor

Benign

Cystadenoma, adenofibroma, cystadenofibroma

Clear cell tumors

Malignant

- Adenocarcinofibroma
- Borderline tumor
- Benign
  - Cystadenoma, adenofibroma, cystadenofibroma
- Transitional cell tumors
  - Malignant
    - Transitional cell carcinoma (non-Brenner type)
    - Malignant Brenner tumor
  - Borderline
  - Benign
    - Brenner tumor
- Squamous cell tumors
  - Squamous cell carcinoma
- Mixed epithelial tumors (specify components)
  - Malignant
  - Borderline
  - Benign
- Undifferentiated and unclassified tumors
  - Undifferentiated carcinoma
  - Adenocarcinoma, not otherwise specified

Sex-cord stromal tumors

- Granulosa-stromal cell tumors
  - Granulosa cell tumor group
    - Adult granulosa cell tumor
    - Juvenile granulosa cell tumor
- Thecoma-fibroma group
  - Thecoma, not otherwise specified
    - Typical
    - Luteinized
  - Fibroma
  - Cellular fibroma

- Fibrosarcoma
- Stromal tumor with minor sex cord elements
- Sclerosing stromal tumor
- Signet-ring stromal tumor
- Unclassified (fibrothecoma)

Sertoli-stromal cell tumors

- Sertoli-Leydig cell tumor group
  - Well differentiated
  - Of intermediate differentiation
    - Variant with heterologous elements (specify type)
  - Poorly differentiated (sarcomatoid)
    - Variant with heterologous elements (specify type)
  - Retiform
    - Variant with heterologous elements (specify type)
- Sertoli cell tumor
- Stromal-Leydig cell tumor
- Sex cord-stromal tumors of mixed or unclassified cell types
- Sex cord tumor with annular tubules
- Gynandroblastoma (specify components)
- Sex cord-stromal tumor, unclassified

Steroid cell tumors

- Stromal luteoma
- Leydig cell tumor group
  - Hilus cell tumor
  - Leydig cell tumor, nonhilar type
  - Leydig cell tumors, not otherwise specified
- Steroid cell tumor, not otherwise specified
  - Well differentiated
  - Malignant

Germ cell tumors

Primitive germ cell tumors

Dysgerminoma

Yolk sac tumor

Embryonal carcinoma

Polyembryoma

Nongestational choriocarcinoma

Mixed germ cell tumor (specify components)

Biphasic or triphasic teratoma

Immature teratoma

Mature teratoma

- Solid
- Cystic
- Fetiform teratoma (homunculus)

Monodermal teratoma and somatic-type tumors associated with dermoid cysts

Thyroid tumor group

- Struma ovarii
  - Benign
  - Malignant (specify type)

Cardinoid group

Neuroectodermal tumor group

Carcinoma group

Melanocytic group

- Malignant melanoma
- Melanocytic nevus

Sarcoma group (specify type)

Sebaceous tumor group

Pituitary-type tumor group

Retinal anlage tumor group

Others

Germ cell sex cord-stromal tumors

Gonadoblastoma

Variant with malignant germ cell tumor

Mixed germ cell-sex cord-stromal tumor

Variant with malignant germ cell tumor

Tumors of the rete ovarii

Adenocarcinoma

Adenoma

Cystadenoma

Cystadenofibroma

Miscellaneous tumors

Small cell carcinoma, hypercalcemic type

Small cell carcinoma, pulmonary type

Large cell neuroendocrine carcinoma

Hepatoid carcinoma

Primary ovarian mesothelioma

Wilms tumor

Gestational choriocarcinoma

Hydatidiform mole

Adenoid cystic carcinoma

Basal cell tumor

Ovarian

Wolffian tumor

Paraganglioma

Myxoma

Soft tissue tumors not specific to the ovary

Others

Tumor like conditions

Luteoma of pregnancy

Stromal hyperthecosis

Stromal hyperplasia

Fibromatosis

Massive ovarian edema

Others

Lymphoid and hematopoetic tumors

Malignant lymphoma (specify type)

Leukemia (specify type)

Plasmacytoma

Secondary tumors

From: Tavassoli FA, Devilee P, eds. World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of the Breast and Female Genital Organs. Lyon: IARC Press; 2003. Used with permission.

Annexure 2
STAGING OF OVARIAN CARCINOMA

FIGO Ovarian Cancer Staging
Effective Jan. 1, 2014

(Changes are in italics.)

STAGE I: Tumor confined to ovaries				
OLD			NEW	
IA	Tumor limited to 1 ovary, capsule intact, no tumor on surface, negative washings/ascites.		IA	Tumor limited to 1 ovary, capsule intact, no tumor on surface, negative washings.
IB	Tumor involves both ovaries otherwise like IA.		IB	Tumor involves both ovaries otherwise like IA.
IC	Tumor involves 1 or both ovaries with any of the following: capsule rupture, tumor on surface, positive washings/ascites.		*IC Tumor limited to 1 or both ovaries*	
			IC1	*Surgical spill*
			IC2	*Capsule rupture before surgery or tumor on ovarian surface.*
			IC3	*Malignant cells in the ascites or peritoneal washings.*

STAGE II: Tumor involves 1 or both ovaries with pelvic extension (below the pelvic brim) or primary peritoneal cancer				
OLD			NEW	
IIA	Extension and/or implant on uterus and/or Fallopian tubes		IIA	Extension and/or implant on uterus and/or Fallopian tubes
IIB	Extension to other pelvic intraperitoneal tissues		IIB	Extension to other pelvic intraperitoneal tissues
IIC	IIA or IIB with positive washings/ascites.			

Old stage IIC has been eliminated

FIGO Ovarian Cancer Staging
Effective Jan. 1, 2014

(Changes are in italics.)

STAGE III: Tumor involves 1 or both ovaries with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes				
OLD			NEW	
IIIA	Microscopic metastasis beyond the pelvis.		*IIIA (Positive retroperitoneal lymph nodes and /or microscopic metastasis beyond the pelvis)*	
			IIIA1	*Positive retroperitoneal lymph nodes only*
			IIIA1(i)	*Metastasis ≤ 10 mm*
			IIIA1(ii)	*Metastasis > 10 mm*
			IIIA2	*Microscopic, extrapelvic (above the brim) peritoneal involvement ± positive retroperitoneal lymph nodes*
IIIB	Macroscopic, extrapelvic, peritoneal metastasis ≤ 2 cm in greatest dimension.		IIIB	*Macroscopic, extrapelvic, peritoneal metastasis ≤ 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen.*
IIIC	Macroscopic, extrapelvic, peritoneal metastasis > 2 cm in greatest dimension and/or regional lymph node metastasis.		IIIC	*Macroscopic, extrapelvic, peritoneal metastasis > 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen.*

STAGE IV: Distant metastasis excluding peritoneal metastasis				
OLD			NEW	
IV	Distant metastasis excluding peritoneal metastasis. Includes hepatic parenchymal metastasis.		*IVA*	*Pleural effusion with positive cytology*
			IVB	Hepatic and/or *splenic parenchymal* metastasis, metastasis to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

ANNEXURE 3

GRADING OF OVARIAN CANCER

As per AJCC – American Joint Committee on Cancer.

Gx – Grade cannot be evaluated

GB – Borderline cancerous

G1 – The tumour is well differentiated

G2 – The tumour is moderately differentiated

G3 to G4 – The tumour is poorly differentiated or undifferentiated

ANNEXURE 4

Scoring system for the ImmunoHistoChemical marker

EGFR and VEGF (Cytoplasmic and / or Membranous Staining)

Percentage:

O/negative – positive staining in <5% of cells

1+ positive staining in 5 to 25% of tumour cells

2+ positive staining in> 25 to <50% of tumour cells

3+ positive staining in >50% of tumour cells

Intensity of staining graded as weak (+), moderate (2+) and strong (3+).

INFORMATION SHEET

Title : Expression of IHC markers EGFR (Epidermal Growth Factor Receptor) and VEGF (Vascular Endothelial Growth Factor) in epithelial ovarian neoplasms

- Your specimen has been accepted.
- We are conducting a study on Epithelial ovarian neoplasms among patients attending Government Kasthurba Gandhi Hospital for women, Chennai and for that your specimen may be valuable to us.
- The purpose of this study is to study the expression of Vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) in epithelial ovarian neoplasms which could thence be used as therapeutic targets in future.
- We are selecting certain cases and if your specimen is found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

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INFORMED CONSENT FORM

Title of the study : "**Expression of IHC markers EGFR (Epidermal Growth Factor Receptor) and VEGF (Vascular Endothelial Growth Factor) in epithelial ovarian neoplasms"**
Name of the Participant :
Name of the Principal (Co-Investigator) :
Name of the Institution : Madras Medical College
Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in the study on "**EXPRESSION OF IHC MARKERS EGFR (EPIDERMAL GROWTH FACTOR RECEPTOR) AND VEGF (VASCULAR ENDOTHELIAL GROWTH FACTOR) IN EPITHELIAL OVARIAN NEOPLASMS".**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the resected soft tissue tumors will be subjected to immunohistochemistry and histopathological examination.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:
Name and signature / thumb impression of the participant (or legal representative if participant
incompetent)
Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

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Master Chart

MASTER CHART

Sl NO	BIOPSY NO	AGE	PROCEDURE DONE	HISTOPATHOLOGICAL TYPE	GRADE	STAGE	EGFR	VEGF
1	1691/12	40	Ovarian Cystectomy	Serous cystadenoma				
2	1692/12	40	TAH With BSO	Serous cystadenofibroma				
3	1533/12	20	TAH With BSO	mucinous cystadenoma				
4	1779/12	23	TAH With BSO	mucinous cystadenoma				
5	1884/12	40	TAH With BSO	mucinous cystadenoma				
6	1912/12	21	Cystectomy	mucinous cystadenoma				
7	1968/12	40	Staging Laprotomy	mucinous cystadenoma				
8	2431/12	32	Cystectomy	Serous cystadenoma				
9	2490/12	55	TAH With BSO	mucinous cystadenoma				
10	827/12	40	TAH With BSO	mucinous cystadenoma				
11	705/12	20	TAH With BSO	mucinous cystadenoma				
12	1095/12	32	TAH With BSO	Serous cystadenoma				
13	462/12	40	TAH With BSO	mucinous cystadenoma				
14	990/12	21	Cystectomy	Serous cystadenofibroma				
15	555/12	20	Cystectomy	Papillary Serous Cystadenoma				
16	567/12	51	TAH With BSO	Papillary Serous Cystadenoma				
17	608/12	40	TAH With BSO	mucinous cystadenoma				
18	1018/12	27	Cystectomy	mucinous cystadenoma				
19	1090/12	40	TAH With BSO	mucinous cystadenoma				
20	513/13	68	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	3	III C	3+	3+
21	2559/13	45	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	2	III B	2+	3+
22	184/13	45	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	3	III C	3+	3+
23	903/13	48	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	3	III C	3+	3+
24	1105/13	58	Staging Laprotomy	Endometrioid adenocarcinoma of ovary	2	II A	3+	3+
25	976/13	63	Staging Laprotomy	Mucinous adenocarcinoma ovary	2	II A	3+	3+
26	2209/13	61	Staging Laprotomy	Clear cell carcinoma ovary	3	III C	3+	3+
27	305/13	58	Staging Laprotomy	Atypical proliferating serous tumor	1		2+	2+
28	405/13	54	Staging Laprotomy	Atypical proliferating serous tumor	1		2+	2+
29	321/13	26	Cystectomy	Mucinous cystadenofibroma				
30	668/13	39	TAH With BSO	Pap serous cystadenofibroma				
31	750/13	51	TAH With BSO	Pap serous cystadenofibroma				
32	1024/13	26	Cystectomy	Mucinous cystadenoma				
33	884/13	50	Staging Laprotomy	Serous cystadenofibroma				
34	1000/13	40	Staging Laprotomy	Serous cystadenofibroma				

Sl NO	BIOPSY NO	AGE	PROCEDURE DONE	HISTOPATHOLOGICAL TYPE	GRADE	STAGE	EGFR	VEGF
35	1063/13	28	Cystectomy	mucinous cystadenoma				
36	1155/13	54	TAH With BSO	mucinous cystadenoma				
37	1300/13	38	Staging Laprotomy	mucinous cystadenoma				
38	1478/13	50	TAH With BSO	Papillary Serous Cystadenofibroma				
39	1919/13	43	TAH With BSO	mucinous cystadenoma				
40	1988/13	46	TAH With BSO	mucinous cystadenoma				
41	2033/13	40	TAH With BSO	Papillary Serous Cystadenofibroma				
42	2116/13	38	TAH With BSO	Papillary Serous Cystadenofibroma				
43	2232/13	36	TAH With BSO	mucinous cystadenoma				
44	35/13	34	TAH With BSO	Papillary Serous Cystadenofibroma				
45	104/14	50	Staging Laprotomy	Micropapillary Serous Cystadenocarcinoma	3	III B	3+	3+
46	700/14	58	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	3	III B	3+	3+
47	2560/14	49	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	2	II A	Neg	Neg
48	205/14	42	Staging Laprotomy	Endometrioid adenocarcinoma of ovary	2	II A	3+	3+
49	305/14	50	Staging Laprotomy	Endometrioid adenocarcinoma of ovary	2	II A	Neg	Neg
50	92/14	45	Staging Laprotomy	Endometrioid adenocarcinoma of ovary	3	II A	3+	3+
51	342/14	76	Staging Laprotomy	Mucinous adenocarcinoma ovary	3	III B	3+	3+
52	428/14	45	Staging Laprotomy	Mucinous adenocarcinoma ovary	1	II A	Neg	2+
53	1088/14	53	Staging Laprotomy	Clear cell carcinoma ovary	3	III C	3+	3+
54	404/14	52	Staging Laprotomy	Adenosquamous carcinoma ovary	3	III C	Neg	Neg
55	1028/14	48	Staging Laprotomy	Atypical proliferating mucinous tumor	1		Neg	1+
56	3143/14	27	TAH With BSO	Mucinous cystadenoma				
57	3293/14	45	TAH With BSO	Serous cystadenoma				
58	3819/14	35	TAH With BSO	mucinous cystadenoma				
59	3943/14	31	TAH With BSO	mucinous cystadenoma				
60	1324/14	31	Ovarian Cystectomy	serous cystadenoma				
61	2358/14	32	Ovarian Cystectomy	Pap serous cystadenofibroma				
62	2521/14	33	TAH With BSO	Serous cystadenoma				
63	2458/14	33	TAH With BSO	mucinous cystadenoma				
64	2624/14	34	TAH With BSO	mucinous cystadenoma				
65	2636/14	45	TAH With BSO	Serous cystadenofibroma				
66	85/14	32	TAH With BSO	Serous cystadenofibroma				
67	222/14	35	TAH With BSO	mucinous cystadenofibroma				
68	750/15	46	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	2	II A	2+	3+
69	1842/15	53	Staging Laprotomy	Papillary Serous Cystadenocarcinoma	2	II B	2+	2+
70	3249/15	58	Staging Laprotomy	Endometrioid adenocarcinoma ovary	3	III C	3+	3+
71	3160/15	66	Staging Laprotomy	Endometrioid adenocarcinoma of ovary	3	III B	3+	3+
72	93/15	60	Staging Laprotomy	Endometrioid adenocarcinoma of ovary	2	II A	2+	2+

Sl NO	BIOPSY NO	AGE	PROCEDURE DONE	HISTOPATHOLOGICAL TYPE	GRADE	STAGE	EGFR	VEGF
73	877/15	70	Staging Laprotomy	Mucinous adenocarcinoma ovary	1	II B	Neg	Neg
74	254/15	70	Staging Laprotomy	Clear cell carcinoma ovary	3	III C	3+	3+
75	1974/15	60	Staging Laprotomy	Clear cell carcinoma ovary	3	III B	3+	3+
76	610/15	45	Staging Laprotomy	Atypical proliferating mucinous tumor	1		Neg	Neg
77	950/15	22	Lap Cystectomy	Papillary Serous Cystadenofibroma				
78	1109/15	57	Staging Laprotomy	Benign Brenner				
79	673/15	27	Cystectomy	Benign mucinous cystadenoma				
80	715/15	41	TAH With BSO	Benign serous cystadenoma				
81	743/15	40	TAH With BSO	Benign mucinous cystadenoma				
82	744/15	54	TAM With B81	Benign mucinous cystadenoma				
83	849/15	24	Ovarian Cystectomy	Benign serous cystadenoma				
84	867/15	48	TAH With BSO	Benign Papillary Serous Cystadenofibroma				
85	52/15	36	TAH With BSO	Pap serous cystadenofibroma				
86	137/15	48	Staging Laprotomy	serous cystadenoma				
87	138/15	50	Staging Laprotomy	serous cystadenoma				
88	381/15	27	Ovarian Cystectomy	mucinous cystadenoma				
89	431/15	33	TAH With BSO	Benign serous cystadenoma				
90	438/15	26	Ovarian Cystectomy	papillary serous cystadenofibroma				
91	955/1	67	Staging Laprotomy	Endometrioid adenocarcinoma of ovary	2	II A	2+	2+
92	942/15	37	TAH With BSO	mucinous cystadenoma				

KEY TO MASTER CHART

TAH with BSO	-	Total Abdominal Hysterectomy with Bilateral Salpingo Oophorectomy
EGFR	-	Epidermal Growth Factor Receptor
VEGF	-	Vascular Endothelial Growth Factor
Neg	-	Negative